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Direct analysis of free base geoporphyrins and metal geoporphyrin complexes by high temperature glass capillary gas chromatography and capillary supercritical fluid chromatography in combination with mass spectrometry

DIRECT ANALYSIS OF FREE BASE GEOPORPHYRINS AND METAL GEOPORPHYRIN COMPLEXES BY HIGH TEMPERATURE GLASS CAPILLARY GAS CHROMATOGRAPHY AND CAPILLARY SUPERCRITICAL FLUID CHROMATOGRAPHY IN COMBINATION WITH MASS SPECTROMETRY

by

Wolfgang Blum

A thesis submitted to the
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Doctor of Philosophy
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Organic Geochemistry Unit, School of Chemistry,
University of Bristol, U.K.

Mai 1989

Capillary Chromatography-Mass Spectrometry Analysis of Geoporphyrins

Dedicated to:

The late father of
glass capillary gas chromatography

Prof. Kurt Grob

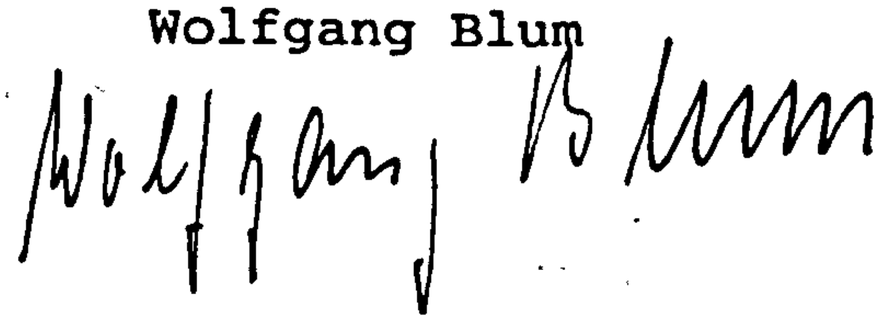
Wir sehen in der Natur nicht Wörter, sondern immer nur Anfangsbuchstaben von Wörtern, und wenn wir alsdann lesen wollen, so finden wir, dass die neuen sogenannten Wörter wiederum bloss Anfangsbuchstaben von anderen sind.

Georg Christoph Lichtenberg
(1793)

Declaration

I hereby certify that the work described in this thesis is my own, except where otherwise acknowledged, and has not been submitted previously for a degree at this, or any other, University.

Wolfgang Blum



C O N T E N T S

PREFACE 1

ACKNOWLEDGEMENT 6

ABSTRACT 7

ABBREVIATIONS 8

GENERAL INTRODUCTION 9

I. THE GAS CHROMATOGRAPHY OF GEOPORPHYRINS 11

I.1. The Gas Chromatography of Free Base and Metalloporhyrins 11

I.1.1. The Unusual Chromatographic Behaviour of Tetrapyrrole Macrocycles 11

I.2. The Gas chromatography of Derivatized Geoporphyrins 15

I.3. The Optimisation of Capillary Gas Chromatography for Geoporphyrin Separation 18

I.3.1. State of the Art in High Temperature Gas Chromatography 18

I.3.2. Direct Analysis of Metal Porphyrin Complexes 19

I.3.2.1. Optimisation of the Column Dimensions and of the Filmthickness 20

I.3.2.2. Selection of the Stationary Phase 21

I.3.2.3. Gas Chromatographic Separation of Metalloporphyrins with Different Central Atoms 29

I.3.3. Analysis of Free Base Porphyrins 30

I.3.4. Selection of the Carrier Gas and Constant Flow Regulation 39

I.3.5. The Use of Specific Atmospheric Detectors 44

II. SUPERCRITICAL FLUID CHROMATOGRAPHY OF PORPHYRINS 46

III. SIMULTANEOUS COUPLING OF HIGH TEMPERATURE GLASS CAPILLARY GAS CHROMATOGRAPHY AND CAPILLARY SUPERCRITICAL FLUID CHROMATOGRAPHY TO A MASS SPECTROMETER 48

III.1. The Coupling of High Temperature Glass Capillary Columns to a Mass Spectrometer 48

III.2. The Coupling of Glass Capillary Supercritical Fluid Chromatography to a High Temperature GC/MS System 51

III.2.1. The Influence of the Mobile Phase on the Ionisation Process 51

IV. THE MASS SPECTROMETRY OF GEOPORPHYRINS 55

IV.1. A Survey of the Literature 55

IV.2. Structure Elucidation of Geoporphyrins by means of Mass Spectrometry 58

IV.2.1. Free Base Porphyrins 58

IV.2.1.1. Electron Impact Ionisation Mass Spectrometry of Free Base Porphyrins 58

IV.2.1.2.	Chemical Ionisation Mass Spectrometry of Free Base Porphyrins	59
IV.2.1.2.1.	Selection of the Chemical Ionisation Reagent Gas	62
IV.2.2.	**Metalloporphyrins**	67
IV.2.2.1.	Electon Impact Mass Spectrometry of Metalloporphyrins	67
IV.2.2.2.	Chemical Ionisation Mass Spectrometry of Metalloporphyrins	72
IV.2.4.	Influence of the Ionisation Mode on the Chromatographic Separation Efficiency in GC/MS	77
IV.2.4.1.	Geoporphyrin Mass Spectra from GC/MS Runs	82
V.	THE ANALYSIS OF OIL SHALE EXTRACTS	84
V.1.	General	84
V.2.	Julia Creek Oil Shale	85
V.2.1.	The Analysis of Julia Creek Oil Shale	87
V.2.1.1.	The First Overview	87
V.2.1.2.	Characterisation of the Individual Liquid Chromatographic Fractions	90
V.2.1.3.	Semi-Systematic Characterisation of the Metalated Geoporphyrins in Fraction JC2, JC3 and JC5	95
V.2.1.3.1.	Fraction JC2	95
V.2.1.3.1.1.	GC/MS Analysis of JC5 on a PS-090 Coated Column	95

V.2.1.3.1.2	GC/MS Analysis of JC 2 on an OV-61-OH Coated Column	110
V.2.1.3.1.3	GC/CIMS Analysis of Fraction JC2	118
V.2.1.3.2.	Semi-Systematic Characterisation of Fraction JC3	123
V.2.1.3.3.	Semi-Systematic Characterisation of Fraction JC4 and JC5	131
V.2.2.	Analysis of Demetallated Free Base Porphyrins of Fractions JC2, JC3 and JC5	139
V.2.2.1	GC/CIMS Investigation of Three C_{32} A-2 Reference Compounds	145
V.2.2.2.	Structure Assignment of Peak 5	160
V.2.2.3.	Assignment of "Unknown" Peak 8 in demetallated JC5	163
V.3.	Capillary SFC/MS Analysis of Fraction JC5-JC7	166
VI.	CAPILLARY COLUMN TECHNOLOGY	171
VI.1	Introduction	171
VI.2.	The Role of Column Technology in Gas Chromatographic Separation of Geoporphyrins	172
VI.3.	Column Technology	172
VI.3.1.	OH/CH_3 O-Terminated Polysiloxane Phases for High Temperature Gas Chromatography	173
VI.3.2.	Wettability	174
VI.4.	Pretreatment of the Glass Surface	175

VI.4.1. Leaching 175

VI.4.2. High Temperature Silylation 175

VI.5. The Condensation Reaction 180

VI.6. The Role of Crosslinking 182

VI.7. Preparation of Glass Capillary Columns for Super-critical Fluid Chromatography 185

VI.5.1. Column Technology 185

VI.8. Resume and Outlook 187

VII. EXPERIMENTAL 191

VII.1. Sample Preparation 191

VII.1.1. Preparation of Hexahydrooctaethylporphyrin 191

VII.1.2. Preparation of Dimethyl-silicon(IV)octaethyl-porphyrin 191

VII.1.3. Preparation of Metallo Octaethylporphyrins 191

VII.1.3.1. Nickel(II)/Co(II)-octaethylporphyrin 192

VII.1.3.2. Copper(II)octaethylporphyrin 192

VII.1.3.3. Vanadium(IV)octaethylporphyrin 192

VII.1.4 Extraction of Sediments 193

VII.1.5 Demetallation of Metalloporphyrins (JC2,JC3,JC5) 193

VII.2. Instruments, Materials and Tools 193

VII.2.1. Gas Chromatography 193

VII.2.3 Combined High Temperature Gas Chromatography / Mass Spectrometry 195

VII.2.3.1. Deactivation of the Interface Glass Capillary Line 195

VII.2.4. Capillary Supercritical Fluid Chromatography 196

VII.2.5. Medium Pressure Column Liquid Chromatographic Fractionation of Julia Creek Crude Extract 197

VII.2.6 Inductively Coupled Plasma Mass Spectrometry 197

VII.2.7 Preparation of Glass Capillary Columns for Gas Chromatography 198

VII.2.7.1. Preparation of High Temperature Stable Columns 198

VII.2.7.2. Increasing the Viscosity of Commercially Available Pre-Polymers 198

VII.2.7.3. Acidic Leaching (Deionisation) 199

VII.2.7.4. High Temperature Silylation 199

VII.2.7.5. Coating and Condensation 200

VII.2.7.6. Preparation of OV-225-OH Coated Columns 200

VII.2.8. Testing Capillary Columns 201

VII.2.8.1 Test Results 202

VII.2.9. Repair of Contaminated Column Entrances by High Temperature Stable Fused-Silica Glass Press-Fit Connections 204

REFERENCES 206

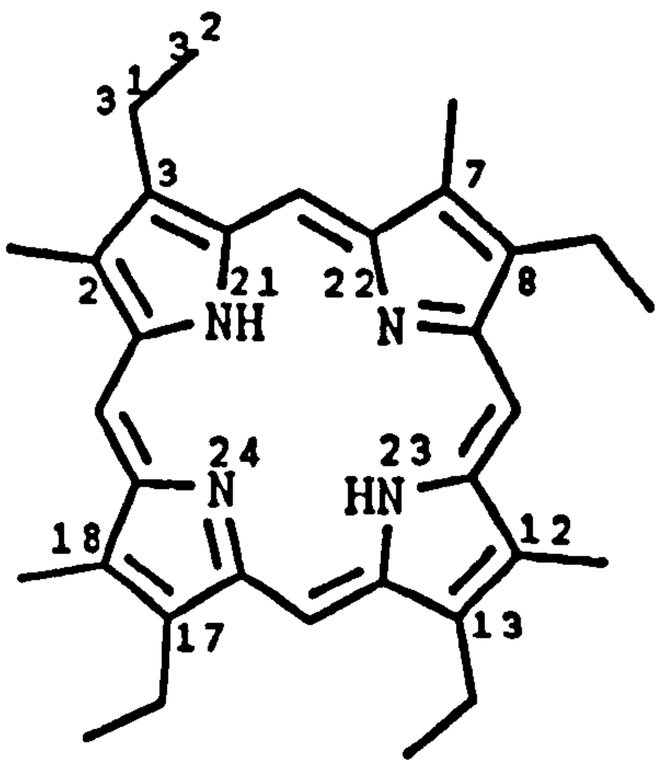
PREFACE

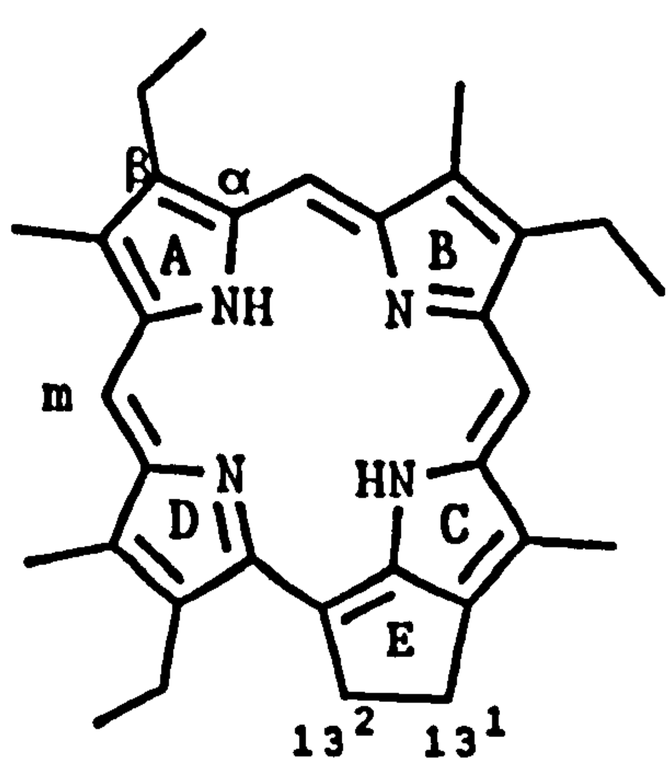
The Thesis is organised into seven chapters:
In Chapter I the capillary gas chromatography of geoporphyrins is reported in general. In this context particular consideration is paid to the advantages of high temperature glass capillary gas chromatography.
Chapter II describes both the device coupling the infant high temperature capillary gas chromatography to a mass spectrometer, and the simultaneous combination of glass capillary supercritical fluid chromatography with a high temperature GC/MS-system.
Chapter III decribes the supercritical fluid chromatography of porphyrins in general.
Chapter IV reviews the mass spectrometry of geoporphyrins. The problems which arise when geoporphyrins are introduced by a capillary column into a mass spectrometer are discussed.
Chapter V describes the GC/MS- and SFC/MS analysis of Julia Creek oil shale extracts. In these applications both the free base and metalloporphyrins were analysed. Moreover, the scope and limitation of geoporphyrin analysis by means of GC/MS and SFC/MS is reflected.
Since this work was only possible owing to recent advances in glass capillary gas chromatography, the underlying column technology which is of fundamental importance for both capillary GC as well as capillary SFC, is finally discussed in chapter VI.
In chapter VII the sample preparation, the instrumental conditions and the analytical tools namely the preparation of high temperature GC- and SFC- capillary columns are presented.
The nomenclature used is generally semi-systematic or based on the IUPAC system. For the discussion of the results particularly in chapter V the semi-systematic nomenclature of Yen et al. (Yen, T. et al.,1969), in which alkylporphyrins are classified by their molecular weight, has been used as modified by Gill (Gill, J.P., 1984a).

Porphyrins with saturated substituents and the same degree of unsaturation as the parent (aetio-)porphyrin, are termed as A class porphyrins. Other porphyrins are classified according to their degree of relative unsaturation: Porphyrins with one exocyclic ring are defined as members of A-2 class, porphyrins with two exocyclic rings as A-4 class etc.

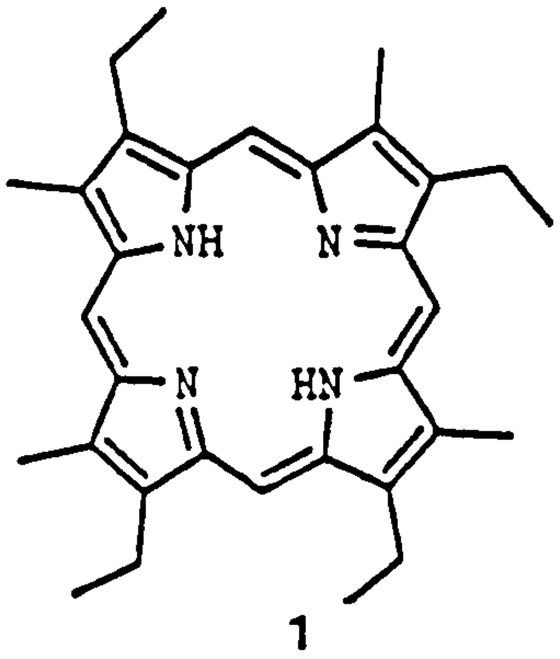
When the structure of a porphyrin is defined, e.g. as in the case of the standard samples, the IUPAC system is used. All skeletal atoms, including the four nitrogens are numbered. Substituent atoms are numbered from their lowest numbered point of attachment to the macrocyle.

For practical purposes, some aspects from the Fischer nomenclature are retained, in particular when a structure could be only partially described. Thus, the carbon atoms are classified as being either α- or ß- to the pyrrole nitrogen, or as being meso-carbon atoms, linking the pyrrole ring. The four pyrrole rings are marked A - D, any additional exocyclic ring is designated by the following letter of the alphabet.



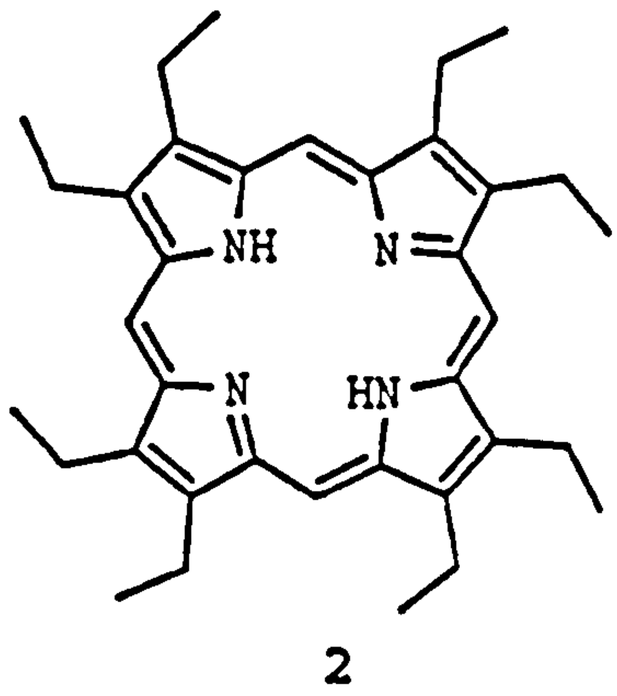


Porphyrin Structures discussed in Chapters 1 - 4



1

2,7,12,18-Tetramethyl-3,8,13,17-tetraethylporphyrin



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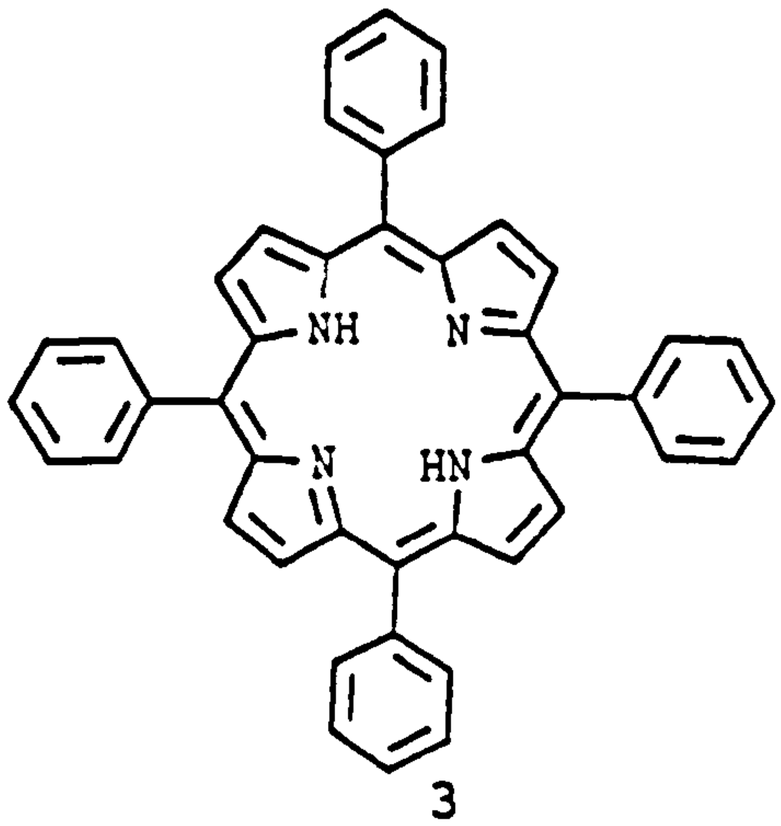

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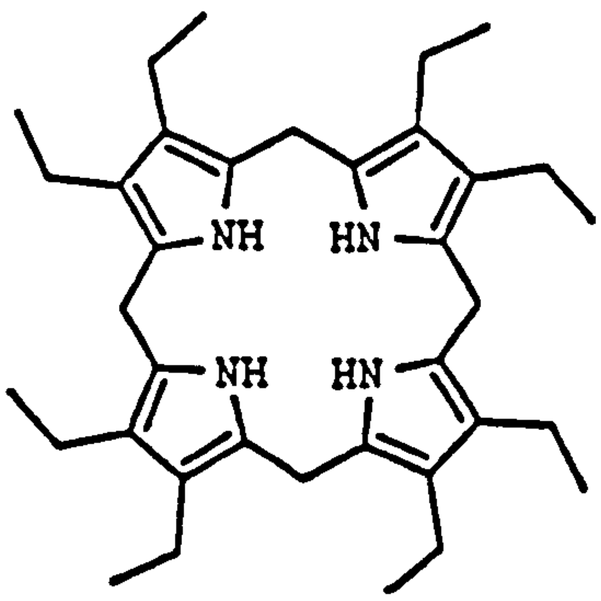

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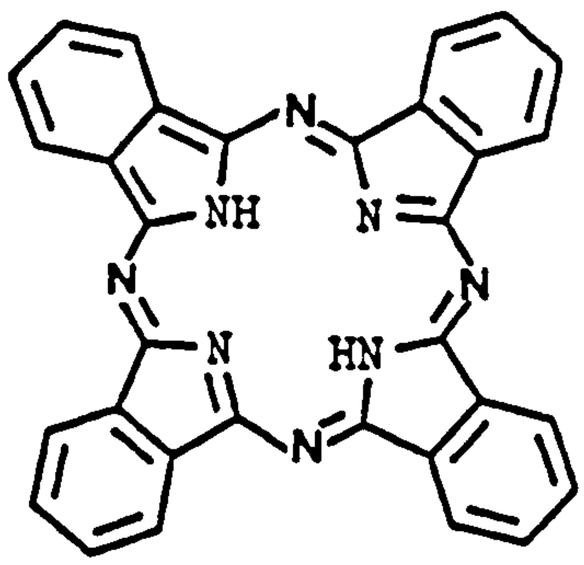
2,3,7,8,12,13,17,18-Octaethylporphyrinogen



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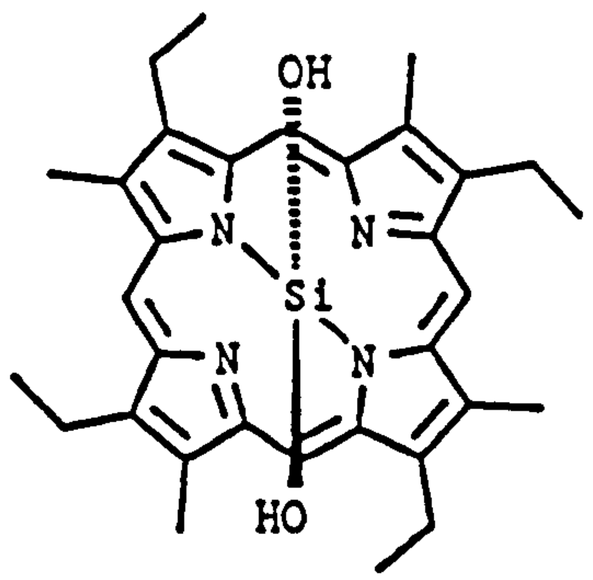

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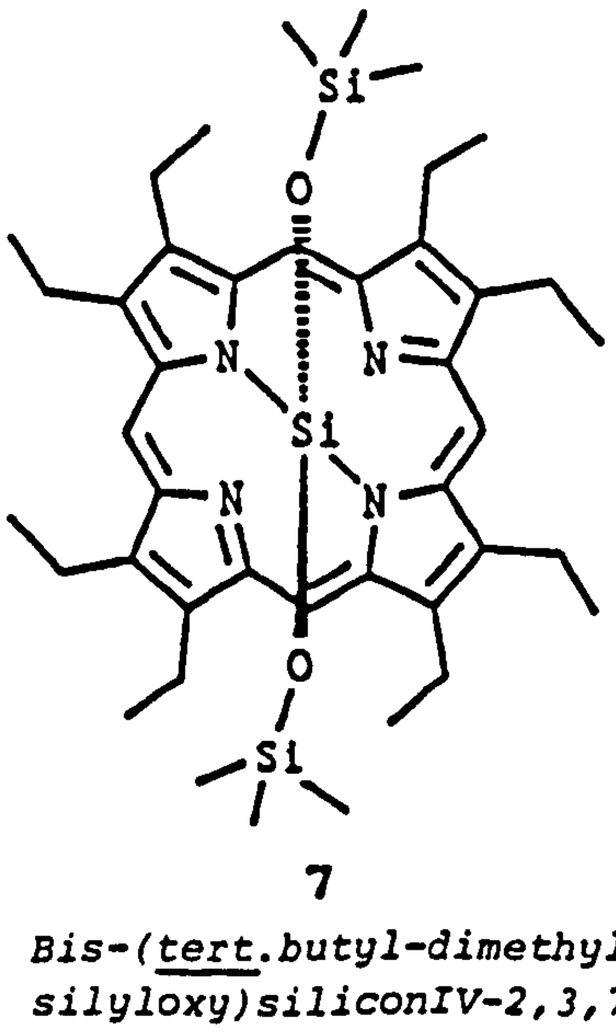
Dihydroxysilicon IV-2,7,12,17-tetramethyl-3,8,13,18-tetra-ethylporphyrin



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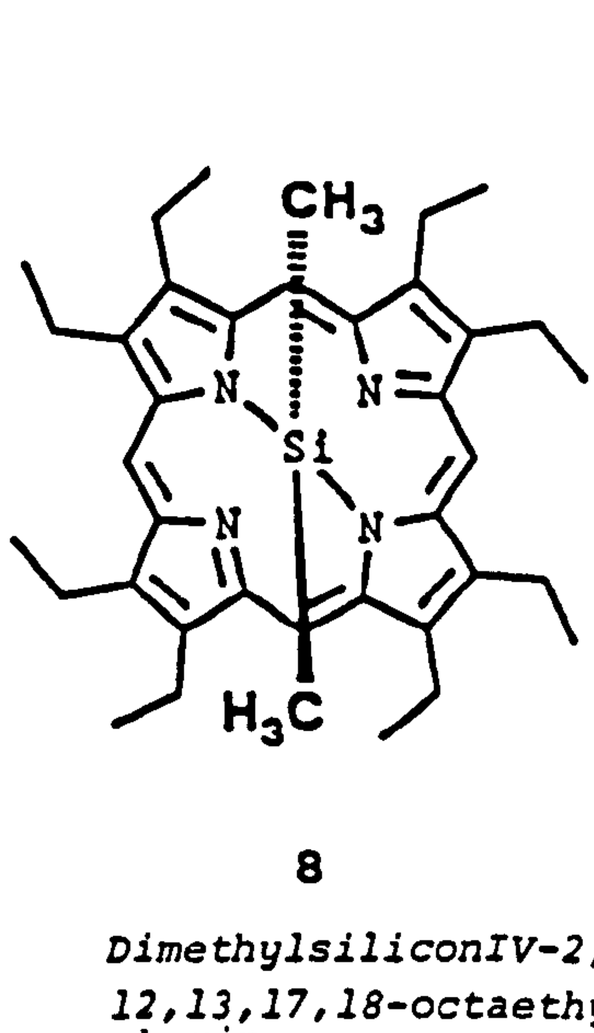
Bis-(tert.butyl-dimethyl-silyloxy)silicon IV-2,3,7,8, 12,13,17,18-octaethylporphyrin



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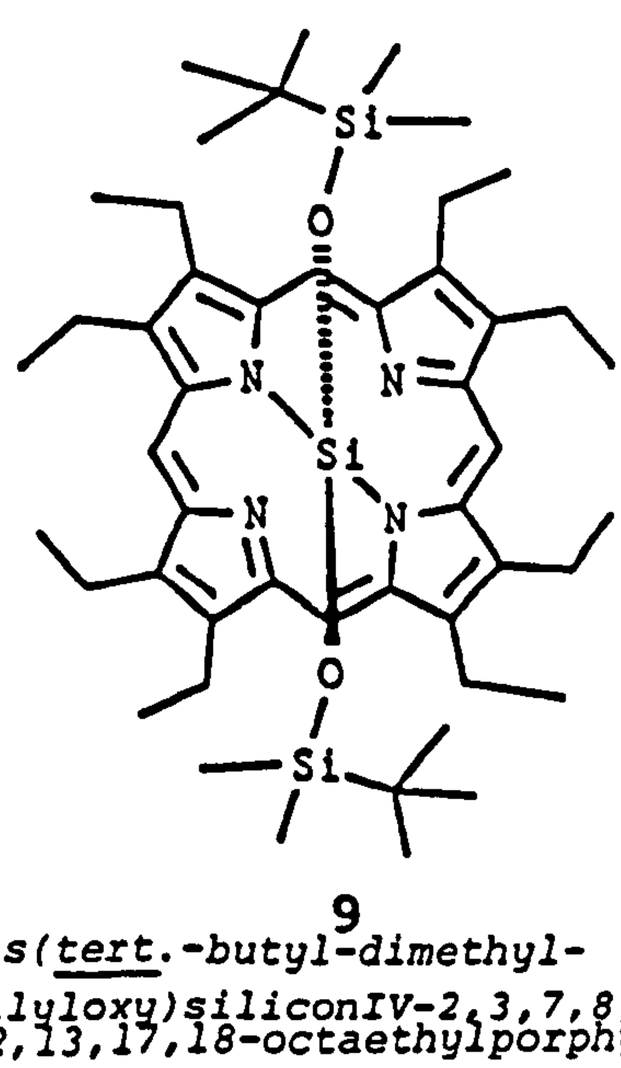
Dimethylsilicon IV-2,3,7,8, 12,13,17,18-octaethylpor-phyrin



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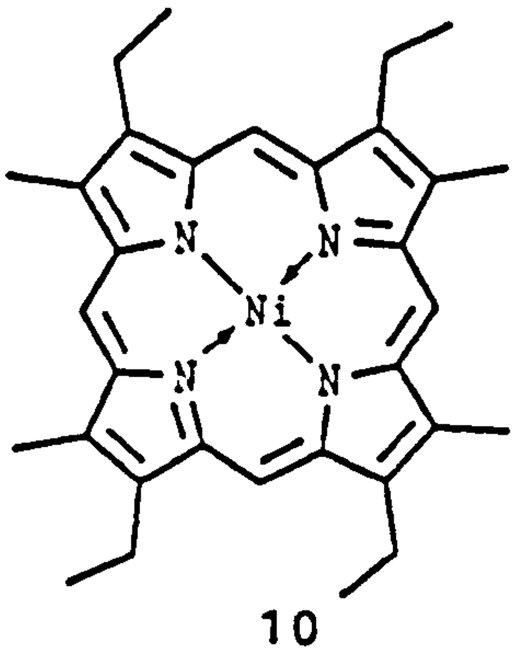

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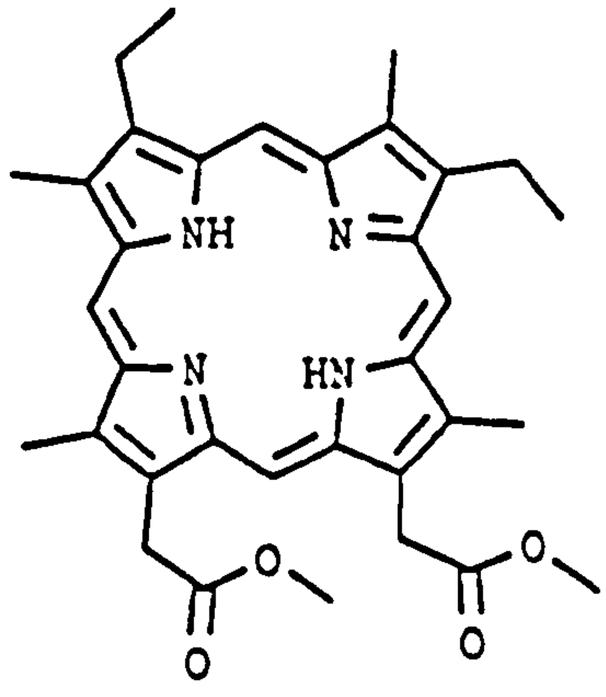
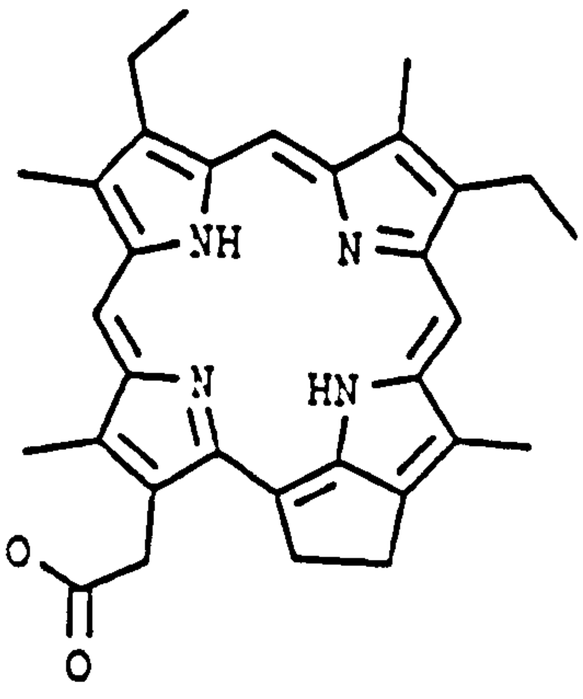
Ni-3,8,13,17-tetraethyl-2,7,12,18-tetramethylporphyrin



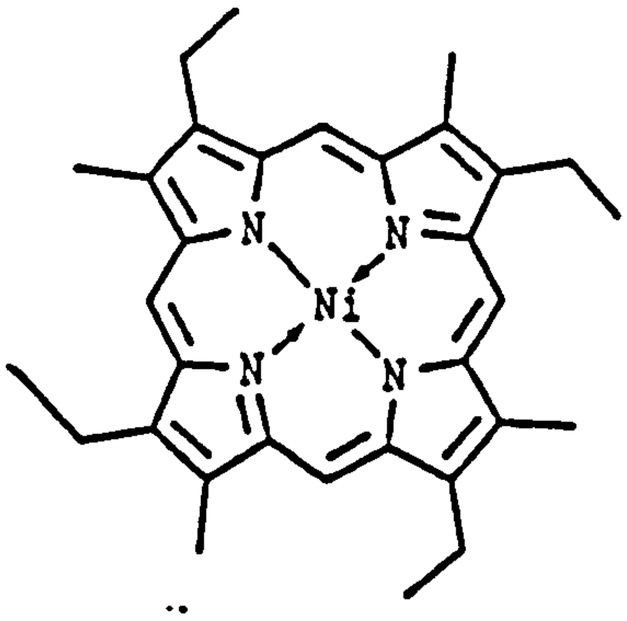
11

13,17,-Bis(2-methoxycarbonylethyl)-3,8-diethyl-2,7,12,18-tetramethylporphyrin



12

17-(2-methoxycarbonylethyl)-13,15-ethano-3,8,diethyl-2,7,12,18-tetramethylporphyrin



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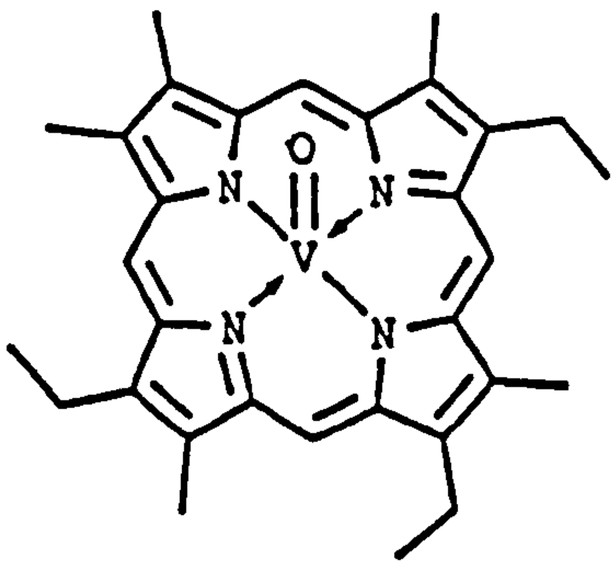
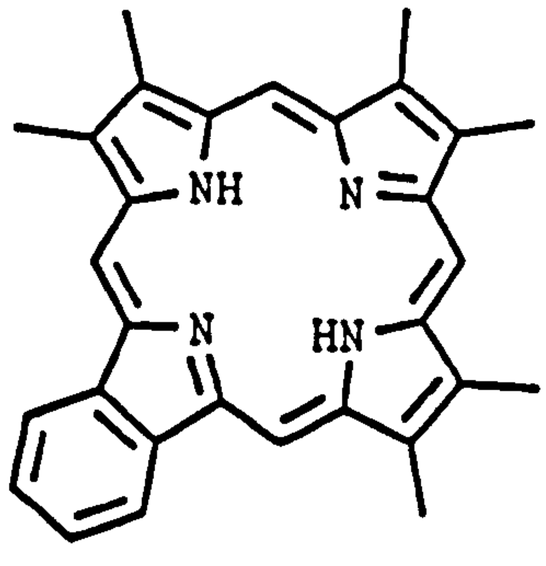
Ni-3,8,13,18-tetraethyl-2,7,12,17-tetramethylporphyrin



14

VO-8,13,18-triethyl-2,3,7,12,17-pentamethylporphyrin



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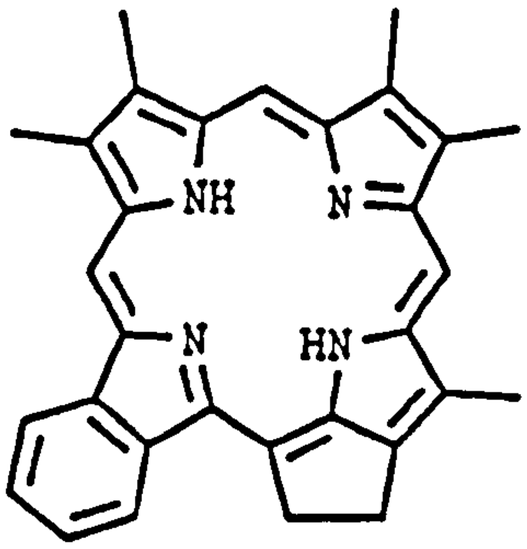
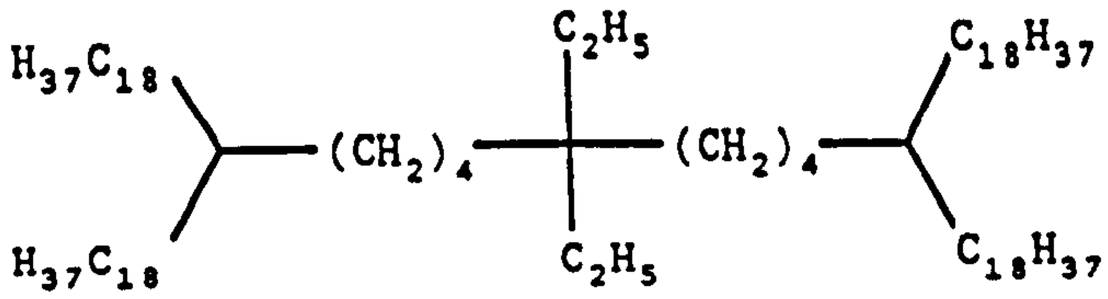
17,18-Benzo-2,3,7,8,12,13-hexamethylporphyrin



16

17,18-Benzo-13,15-ethano-2,3,7,8,12-pentamethylporphyrin



17

24,24'-diethyl-19,29-dioctadecylheptatetracontane

Some aspects of this work, particularly those concerning high temperature gas chromatography, have already been published:

i High Temperature Glass Capillary Gas Chromatography Using OH-Terminated Polysiloxane Stationary Phases. Separation of Antioxydants and UV-Stabilizers.
W. Blum and L. Damasceno, HRC & CC, 10 (1987) 472

ii Glass Capillary Gas Chromatography-Mass Spectrometry at High Temperatures. Direct Analysis of Free Base Porphyrins and Metal Porphyrin Complexes Extracted from the Serpiano Oil Shale.
W. Blum, W.J. Richter and G. Eglinton, HRC & CC, 11(1988)148

iii Simultaneous Coupling of Capillary Supercritical Fluid Chromatography and High Temperature Glass Capillary Gas Chromatography to a Mass Spectrometer.
W. Blum, K. Grolimund, P.E. Jordi and P. Ramstein
HRC & CC, 11 (1988) 441

iv Säulen für die Gaschromatographie.
W. Blum, Nachrichten aus Chemie, Technik und Laboratorium, 36 (1988) 165

v Preparation of High Temperature Stable Glass Capillary Columns Coated with PC-090 (20% Diphenyl Substituted CH_3O-Terminated Polysiloxane). A Selective Stationary Phase for the Direct Analysis of Metalloporphyrin Complexes.
W. Blum and G. Eglinton, HRC & CC, 12 (1989) 290

vi Glass Capillary Gas Chromatography-Alkali Flame Ionisation Detection at High Temperatures. Direct Analysis of Free Base Porphyrins.
W. Blum and G. Eglinton, HRC & CC, 12 (1989) 621

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Dr. Pat Sandra (University of Ghent) is thanked for a sample of medium polar, high temperature stable OH-terminated polysiloxane phase (OV-61-OH).

This thesis was initiated by my late friend Prof. Kurt Grob. I have tried to do it in his spirit.

ABSTRACT

This Study describes the direct structure analysis of naturally occurring geoporphyrin mixtures, either as metal complexes or as demetallated free bases extracted from different oil shales. Continuing a series of related investigations at the University of Bristol (Alturki 1972; Hajlbrahim 1986; Shaw 1981; Quirke 1983; Wolff 1983; Gill 1984; Chicarelli 1985 and Kaur 1987) it is intended to emphasise the prerequisites of direct geoporphyrin analysis as shown in part five, including the analytical tools and the underlaying technology. Geoporphyrins serve as an example (independent of their geochemical relevance) to demonstrate on one hand the lasting significance of glass capillary gas chromatography and on the other hand the possible extension of the latter by capillary supercritical fluid chromatography, particularly in combination with mass spectrometry. These optimised hyphenated techniques have been succesfully applied in the direct analysis of different oil shale extracts, and the structures of a number of porphyrins could be corroborated or partially elucidated by means of high temperature capillary GC or SFC in direct combination with EI and CI mass spectrometry.

GLOSSARY AND ABBREVIATIONS

A class
A-2 class
A-4 class see page 3-4
A-6 class
A-7 class

aetio	- aetioporphyrin (see page 3)
amu	- atomic mass units
CI	- chemical ionisation
CPTMDS	- bis(cyanopropyl)tetramethyldisiloxane
DPED	- deoxophylloerythroporphyrin
DPTMDS	- diphenyltetramethyldisilazane
DMTMDS	- dimethyltetramethoxydisiloxane
EI	- electron impact (ionisation)
Et	- ethyl
FID	- flame ionisation detector
GC	- gas chromatography
GC/MS	- gas chromatography / mass spectrometry
HPLC	- high pressure liquid chromatography
HTGC	- high temperature gas chromatography
ICPMS	- inductively-coupled plasma mass spectrometry
LC	- liquid chromatography
Me	- methyl
MID	- multiple ion detection
MPLC	- medium pressure liquid chromatography
MSA	- methane sulphonic acid
MS	- mass spectrometry
NMR	- nuclear magnetic resonance (spectroscopy)
OEP	- octaethylporphyrin (see page 3)
SFC	- supercritical fluid chromatography
TMSO	- trimethylsilyloxy
TDMSO	- tert-butyldimethylsilyloxy
UV/Vis	- ultra-violet and visible
vanadyl	- oxovanadium IV

GENERAL INTRODUCTION

The occurrence of metalloporphyrins in the organic matter of sediments, their origin from biogenic precursors as well as the conversion of chlorophyll-a and haem into metal porphyrin complexes (geoporphyrins), was first proposed by Treibs (Treibs, A., 1934, 1936).

But, owing to the lack of suitable analytical techniques, it needed several decades till full or partial structure assignments of individual geoporphyrins in complex mixtures of naturally geoporphyrins became feasible.

Structure analysis of geoporphyrin extracts requires "multidimensional" analytical techniques. In order to master the mixture situation to some extent Thomas and Blumer (Thomas, W. and Blumer, M., 1964) started with thin layer chromatographic (TLC) separation of crude extracts in individual fractions followed by a mass spectrometric (MS) characterisation. Later on Eglinton et al. (Eglinton, G. et al., 1979a) replaced TLC by high pressure liquid chromatography (HPLC) and in combination with nuclear magnetic resonance spectroscopy (NMR) and mass spectrometry (MS) they elucidated the structures of four individual geoporphyrins from an oil shale. Since then, this off line approach has become the most successful way to assign the structures of unknown geoporphyrins.

The use of on line coupled chromatographic/spectrometric methods in the analysis of geoporphyrins became possible after Boylan and Calvin (Boylan, D.B. and Calvin, M., 1967) described the conversion of free base porphyrins, which at this time could not be tackled by gas chromatography, into more volatile bis(trialkylsilyloxy)siliconIV-derivatives. These derivatives were adapted to capillary gas chromatography by Alexander et al. (Alexander, R. et al. 1980), and the first application by using direct combination of capillary gas chromatography/mass spectrometry (GC/MS) was just a question of time. It was first realised by Marriot

(Marriot, P.J. et al., 1984). Using an artificial standard mixture, the direct analysis of underivatised free base and metallo-alkylporphyrins in principle was shown to be possible as well (Marriot, P.J. et al., 1982). The first high resolution gas chromatographic analysis of naturally occurring geoporphyrins from Serpiano oil shale, by means of 20m high temperature glass capillary columns combined with a mass spectrometer, was reported by Blum (Blum, W. et al. 1988a).

This study continues the discussion about the merits and limitations of high temperature glass capillary GC and its logical extension, capillary supercritical fluid chromatography (SFC) in the direct analysis of both, free base- and metallogeoporphyrins. It was tried to optimise the parts of the hyphenated analytical techniques as individual analytical methods. They influenced each other after coupling, but at last an useable analytical tool for the direct mixture analysis of geoporphyrins has been obtained.

I. THE GAS CHROMATOGRAPHY OF GEOPORPHYRINS

I.1. The Gas Chromatography of Free Base and Metalloporphyrins

Although geoporphyrins, which exist either as free bases or metalloporphyrins, are thermally stable, they were up to the early 1980's considered not to be separable by capillary gas chromatography. The first capillary gas chromatograms were obtained by Marriot (Marriot, P.J et al. 1982) and later by Gill (Gill,J.P., 1984) by the use of short pieces of 4-6 m fused silica capillaries coated with an apolar polysiloxane phase (OV-1). Though these authors demonstrated that the gas chromatographic analysis of this class of compounds was in principle feasible, apart from two reports only a few evaluations of gas chromatographic properties of alkylporphyrins have been attempted (Roberts, J. et al. 1983; Gallegos, E. et al., 1985), due to the insufficient temperature stability of commercially available capillary columns. The first high resolution gas chromatographic analysis of naturally occurring crude extracts from Serpiano oil shale, by means of 20m glass capillary columns coated with medium polar high temperature stable OH-terminated polysiloxanes, was reported by Blum (Blum, W. et al. 1988a).

I.1.1. The Unusual Chromatographic Behaviour of Tetrapyrrole Macrocycles

Several mechanisms by which tetrapyrroles associate with themselves and with other molecules have been described and were carefully reviewed by Gill (Gill, J.P. 1984). Since any type of association will contribute to the low-volatility of porphyrins it will also influence chromatographic behaviour. Fig.1 shows the chromatogram of an artificial mixture of three free-base porphyrins and two aliphatic hydrocarbons.

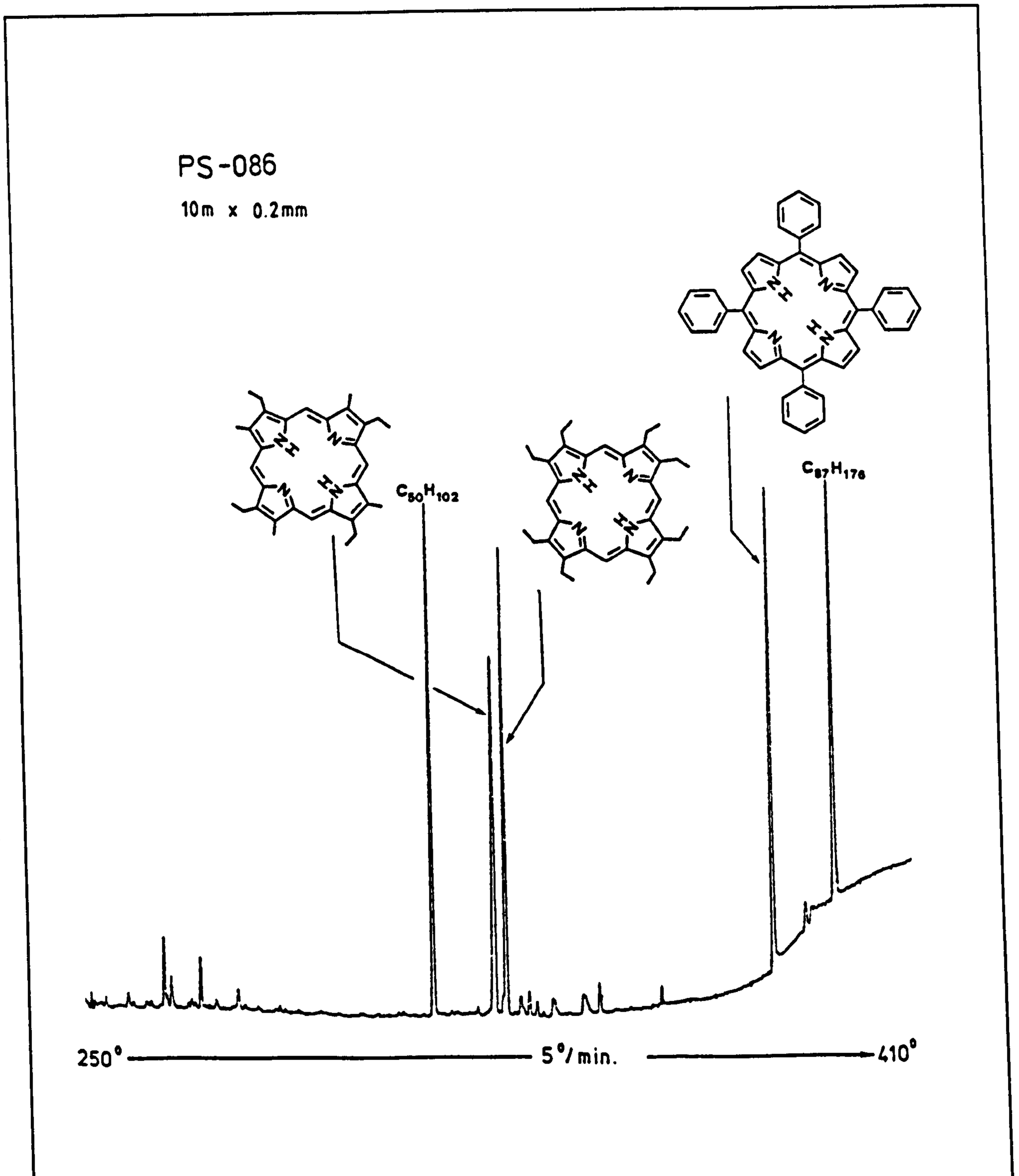


Fig.1 FID chromatogram of pentacosane; 2,7,12,18-tetramethyl-3,8,13,17-tetra-ethylporphyrin (etio III); 2,3,7,8,12,13,17,18-octaethylporphyrin; 5,10,15,20-tetraphenylporphyrin and "apolan" ($C_{87}H_{176}$).

Although these three alkylporphyrins aetio-porphyrin-III (1), octaethylporphyrin (2) and meso-tetraphenylporphyrin (3) have only molecular weights between 478 and 614 daltons their retention temperature was found to be in a range between n-pentacosane ($C_{50}H_{120}$, M=702) and the branched hydrocarbon $C_{87}H_{176}$, M=1220, "apolan" (17) (Zeltner, P. et al. 1979).

Starting from the principle that whenever a chromatographic analysis is carried out on an apolar column, "quasi-chemical" interactions (e.g. Van der Waals forces, quasi-hydrogen bonds etc.) of the compound to be chromatographed and the functional groups of the stationary phase can be excluded, the retention behaviour of a compound then depends only and directly on its vapour pressure and therefore on its molecular weight (Wehrli, A. et al., 1959).

Since the alkylporphyrins appear at unexpectedly high retention temperatures, it can be deduced that they probably elute as molecules having higher molecular weights than expected, because they tend to form dimers via self-association, even when diluted in the stationary phase [other authors paraphrased this behaviour as "association phenomena" (Marriot, P.J. et al., 1984) or as "intermolecular aggregative interactions" (Gill, J.P., 1984c)].

The very high retention temperature of geoporphyrins also intimates that on one hand they are well soluble in the stationary phase whereas, on the other hand, their residence time in the mobile (gas) phase is negligible.

This supposition of a feasible dimerisation is in agreement with NMR measurements carried out in solution. From these investigations, on both free base porphyrins and their metal complexes, the existence of dimeric species was proposed (Abraham, R.J et al., 1966; Doughty, D.A. et al., 1969) which form layered structures where the macrocycles are stacked above each other. This concept seems to disagree with MS-detections of the respective GC-peaks, which gave mass spectra of monomers (Blum, W. 1988a, moreover, neither in positive nor in negative ionisation mode, e.g. of octaethylporphyrin, could dimers be observed).

The contradiction can be explained by the fact that in contrast to the chromatographic process, the ionisation occurs in the gas-phase where the monomers are predominant. In order to get an impression of the "real" retention temperature of a monomeric alkylporphyrin a simple experiment was carried out to destroy the aromaticity of the tetrapyrrole macrocycle and its capability to undergo self-associations.

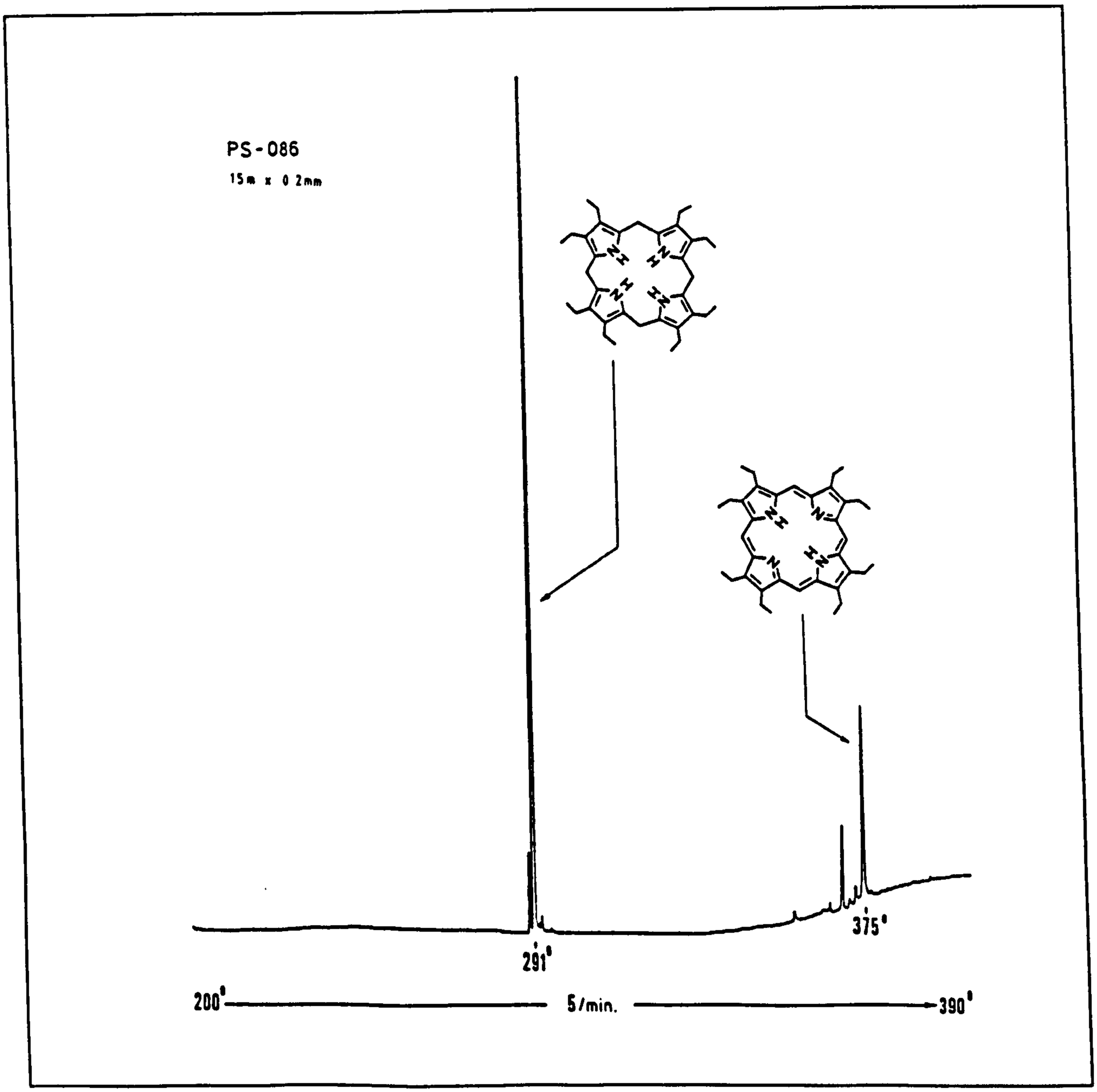
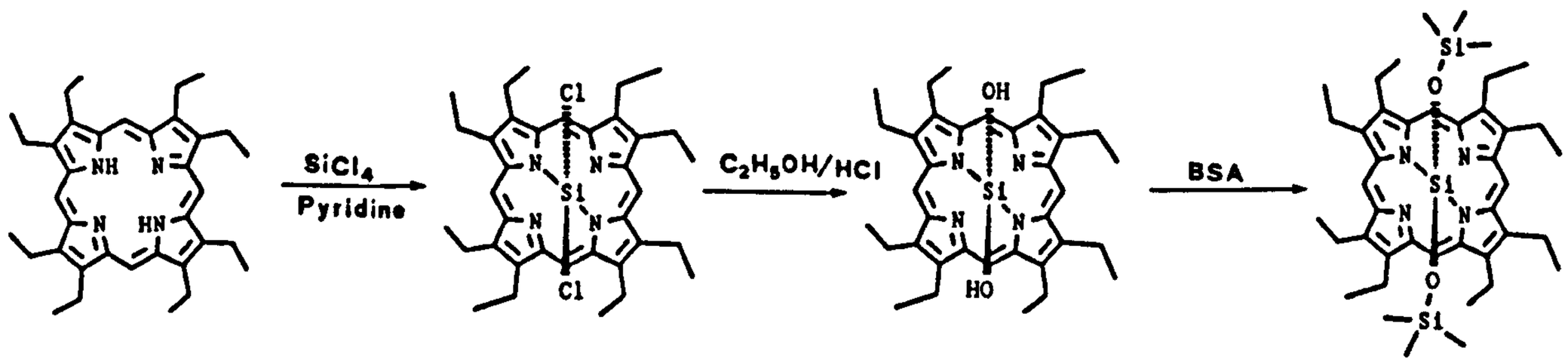


Fig.2 FID gas chromatogram of the free base of octaethylporphyrin and of its corresponding hexahydroderivative (porphyrinogen). For experimental details see Section VII.1.1.

After hydrogenation of free-base octaethylporphyrin (2) with hydrogen and Raney-Ni as a catalyst (Budzikiewicz, H. et al.1976, for experimental details see Section VII.1.1.) the respective porphyrinogen (4) appears at a temperature of only 291°C, that means in the normal range of a compound with a molecular weight of 542 dalton, whereas the fully conjugated porphyrin eluted at a temperature of 375°C. Moreover, the porphyrinogens eluted at similar retention temperatures as the standard derivatives in geoporphyrin mixture-analysis, the bis(trialkylsilyloxy)silicon IV compounds. Therefore it can be deduced that the gas chromatography of both the underivatised free-base porphyrins and their metal complexes should be classified as the chromatography of the respective dimers having effective molecular weights around 1000, whereas the standard procedure for geoporphyrin fingerprinting is dealing with the respective monomers.

I.2. The Gas Chromatography of Derivatized Geoporphyrins

The gas chromatographic analysis of geoporphyrins became possible after Boylan and Calvin (Boylan,D.S. et al.,1967) described a method to prepare volatile porphyrin derivatives by converting free-base alkylporphyrins into bis(trialkylsilyloxy)silicon IV derivatives, thereby ensuring that they behaved as more suitable monomers. They adapted an approach which was reported to produce silicon phthalocyanins (5) by direct reaction of the free-bases with tetrachlorosilane (Barrett, P.A. et al., 1936). Although this method failed for phthalocyanins, it proved to be successful in the preparation of silicon porphyrins, and is still the basis of the preparation of gas chromatography-capable porphyrin derivatives. In Boylan and Calvin's original report tetrachlorosilane was added to aetioporphyrin (1) in dry pyridine and heated in a sealed glass tube at 185°C for 6 hours. After a subsequent hydrolysis with ethanol/HCl the dihydroxysilicon IV aetio-porphyrin (6) is produced. Treatment of this compound with bis(trimethylsilyl)acetamide (BSA) gave the expected bis(trimethylsilyloxy)silicon IV aetioporphyrin (7).



Scheme 1: Reaction sequence employed by Boylan and Calvin for the preparation of bis(trimethylsilyloxy)silicon IV octaethylporphyrin (7).

This derivative showed a higher volatility and eluted at a significant lower retention temperature than the underivatised free-base porphyrin. We discuss this approach again in order to prove whether the silicon insertion or the subsequent silylation of the two hydroxy ligands is responsible for the higher vapor pressure. For this purpose a silicon insertion was accomplished using dichlorodimethylsilane at the described conditions (see VII.1.2) The result of this reaction is a stable derivative (8) with a silicon atom in the centre of the treated free base octaethyl-porphyrin (5) but, as shown in Fig.3 below, eluting at a higher retention temperature than the corresponding free base. This implies that an inserted silicon atom modifies the retention temperature in the same way as any other chelating atom and therefore decreases the volatility. Only if the ligands at the central atom are bulky enough to avoid aggregation of the two macrocycles, will a monomerisation, manifesting itself in a reduced retention temperature, be observed.

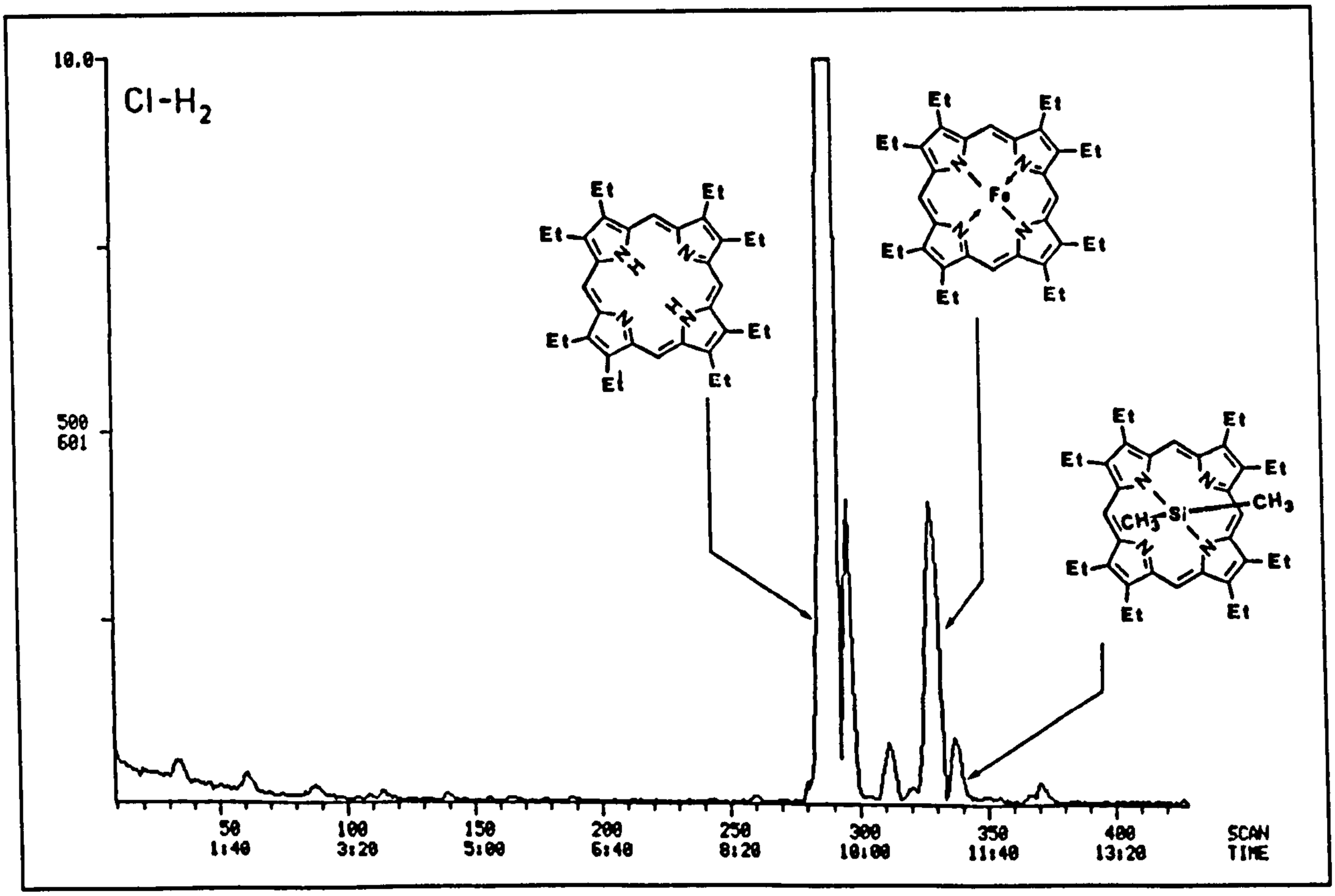


Fig.3 Selected CI/H_2-TIC ion chromatogram (m/z 500-600) of octaethyl porphyrin and the corresponding dimethyl-silicon IV derivative. Iron porphyrin must have been produced during preparation and handling of the sample.

Some years later Boylan and Calvin's initial approach was adapted to capillary gas chromatography by Alexander (Alexander, R. et al., 1980) and, in combination with mass spectrometry, a number of naturally occurring geoporphyrin mixtures from various sources could be characterised (Marriot, P.J. et al., 1984; Eglinton, G. et al., 1985; Gill, J.P. et al., 1984b; Hein, C.S. et al., 1985; Gill, J.P. et al., 1986) on fused silica capillary columns of 10-20 m length, coated with apolar polysiloxane gums (OV-1 and OV-73). The columns had a maximum working temperature of about 300°C. Since Alexander's first report, in all following investigations neither the selectivity nor the efficiency of the columns were changed in order to improve the resolution, and no further optimization has been attempted.

I.3. The Optimisation of Capillary Gas Chromatography for Geoporphyrin Separation

I.3.1. State of the Art of High Temperature Gas Chromatography

Although the term "high temperature" was never exactly defined in this context, it is common linguistic usage, derived from practical experience, that high temperature gas chromatography starts at 350°C whereas the upper working temperature remains open (at present about 450°C). The preparation of high temperature stable apolar and medium polar glass capillary columns using the advantages of the condensation process of terminal silanol groups of OH-terminated polysiloxane phases and residual silanol groups of the deactivated support surface was first reported by Grob and Grob (Grob, K. and Grob, G., 1985) and later by Blum (Blum, W., 1985). But despite the fact that since then further advantages have been published (Lipsky, S., 1986a,b;Blum, W. et al.,1987; Blum, W. et al., 1988a; Blum and Eglinton, 1989; Sandra, P. et al., 1988 and references cited therein) many analysts are obviously still reluctant to use high temperature capillary gas chromatography for the separation of middle mass molecules (molecules with a molecular weight around 1000) even if the compounds are thermostable. It is the merit of Lipsky et al. (Lipsky, S. et al., 1986a,b) that they stressed the insufficient thermal stability of polyimide-clad, fused-silica capillaries which dominate as support material in capillary gas chromatography. Two main drawbacks were pointed out. First, polyimide-coated, fused-silica capillaries tend to break spontaneously after prolonged exposure to temperatures above 300°C. Secondly, their upper temperature limit is lower than that of some polysiloxane phases. In other words, for the first time in gas chromatography the working range of a column is more likely limited by the temperature stability of the support than by the degradation temperature of the stationary phase. Lipsky et al. preferred aluminium-clad, fused-silica

capillaries to overcome the above limitations. A systematic comparison between fused-silica and borosilicate-glass as a support has not been undertaken yet and the question as to which support is superior remains open. However the economic advantages as well as the availability of borosilicate glass favour this material for tailor-made columns (Grob, K., 1985; Rohwer, E. et al.,1987); such columns are of particular interest when the mixture situation is as complex as in the case of geoporphyrins. As emphasised in earlier reports (Blum,W.,1985; Grob,K. and Grob,G.,1985; Blum, W.,1986b) the primary advantage of OH-terminated phases is the outstanding inertness and thermostability. The use of this class of polymers is therefore a prerequisite of any high temperature capillary gas chromatography, irrespective of whether glass- or metal-clad, fused-silica columns are used. This point was discussed in two communications (Lipsky,S.,1986; Blum,W.,1986c). Details of the underlying technology and the preparation of the columns are given in chapters six and seven.

I.3.2. Direct Analysis of Metal Porphyrin Complexes

The direct analysis of untreated geoporphyrin extracts by means of high temperature gas chromatography (combined with mass spectrometry, see Section V) would have certain advantages. Direct determination of the molecular weight and therefore the homologue distribution within a mixture is in principle possible, and both free-base and metalloporphyrins can be determined even in trace amounts and even if they just differ in the chelating metal. Furthermore, since in some dichloromethane crude extracts the porphyrins elute significantly later than the unavoidable cluster of hydrocarbons, which was found in most of the oil shales, it is not necessary to purify the crude extracts prior to the analysis in some cases (Blum, W., et al., 1988a). Thus, the method is fast and moreover, comparably little quantities of oil shales are needed.

I.3.2.1. Optimisation of the Column Dimensions and of the Film thickness

Owing to the extreme complexity of the most of the geoporphyrin extracts the highest possible separation efficiency is required. But for reasons discussed recently (Blum,W. et al., 1988a), the margin for the possibilities for optimisation of the column dimensions and phase thickness is rather limited. Particularly in the analysis of metalloporphyrins, which elute about 20°C later than the corresponding free bases, the maximum working range of a column is needed. If porphyrins shall be analysed in the range of the temperature program the column length is limited to 15-20 m, (depending on the source and the nature of a given oil shale extract), provided hydrogen is used as a carrier gas, and the gas flow is kept constant over the whole temperature range (see Section I.3.4.). The pronounced tendency of porphyrins to absorb on active surfaces was found to be critical. Thus the film thicknesses of the coatings are of some importance due to the masking property of the stationary phase. It should not be decreased below 0.15 µm, especially because the durability and the dynamic range of a column was found unacceptable with thinner films.

On-column injection is a prerequisite for any type of geoporphyrin analysis. Therefore, the inside diameter of a column cannot be narrowed down too far. Using syringe needles of 0.17 mm outer diameter, the column inside diameter can be decreased to 0.2 mm. For the analysis of bis(trialkylsilyloxy)-SiIV-derivatives columns of 20 m x 0.2 mm, coated with a film of 0.15 µm thickness were preferred, whereas for the analysis of the free-base and the metalloporphyrin extracts, due to the higher retention temperatures which lead to a strong pressure decrease if the inside diameter is too narrow, columns with an i.d. of 0.3 mm gave better results.

I.3.2.2. Selection of the Stationary Phase

High resolution capillary gas chromatography of untreated oil shale extracts requires working temperatures around 400°C to elute the porphyrins (Blum, W. et al., 1988a). This first condition already confines the variety of suitable stationary phases. For reasons stated in Section VI.2.1., only commercially available polymers are used in this study. Both conditions are met only by the five following phases:

a. PS-347.5 (100% methyl, OH-terminal)
b. PS-089 (95% methyl, 5% phenyl, OH-terminal)
c. PS-086 (85% methyl, 15% phenyl, OH-terminal)
d. PS-090 (80% methyl, 20% phenyl, CH_3O-terminal)
e. OV-61-OH (67% methyl, 33% phenyl, OH-terminal)

The comparison of the following five chromatograms of a dichloromethane extract of column liquid chromatographic fraction of Marl slate oil shale (Ni-porphyrin fraction) demonstrates that the separation of metallo-alkylporphyrin isomers and homologues is good on the most apolar column (trace 4a) and the most polar column (trace 4e). On both columns about 30 separated fractions could be observed, although on each column, owing to the different selectivity different pairs of metallo-porphyrins were resolved. Probably due to π-interactions between the phenyl substituent of the stationary phase and the extended π-electron system of the porphyrin skeleton, on the column coated with OV-61-OH (33% phenyl) porphyrins with additionally exocyclic rings elute later and the highest selectivity for metallo-porphyrins could be achieved. On the other hand this coating also shows higher retention temperatures for all porphyrins. As discussed in section I.3.4, the separation number values (TZ-values, Kaiser, R., 1969) of a column are inversely proportional to the retention temperatures. Therefore, the resolution of the porphyrin cluster as a whole is partially

compensated by the loss of separation efficiency, despite the superior selectivity of the more polar column. In practice, for the analysis of metalloporphyrins the use of both columns is recommended since homologues and isomers have to be separated. Thus, in the FID chromatograms Fig.4a and 4e it is demonstrated that a nickel porphyrin fraction of Marl slate oil shale, using the merits of an apolar- and a medium polar polysiloxane phase, respectively, is resolved into more than forty single porphyrin GC peaks. However, in order to get a reliable comparison among those traces GC/MS measurements are necessary.

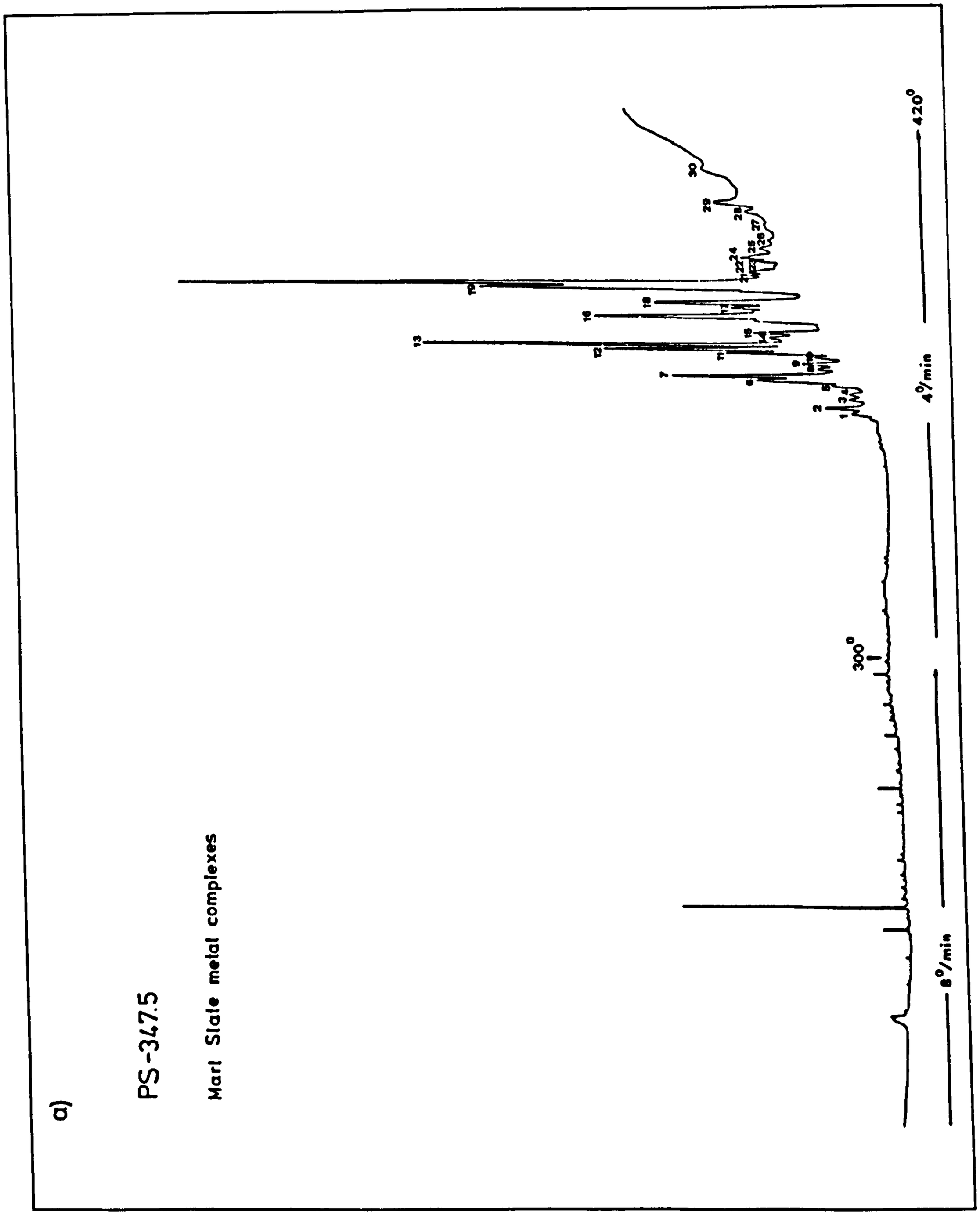


Fig.4a FID-chromatogram of a chloroform extract of a preseparated Marl Slate oil shale analysed on a 20m x 0.3mm, PS-347.5 coated column.
In this and other Figs. a number is given to each peak in sequence from low to high retention temperatures. Each trace is separately numbered.

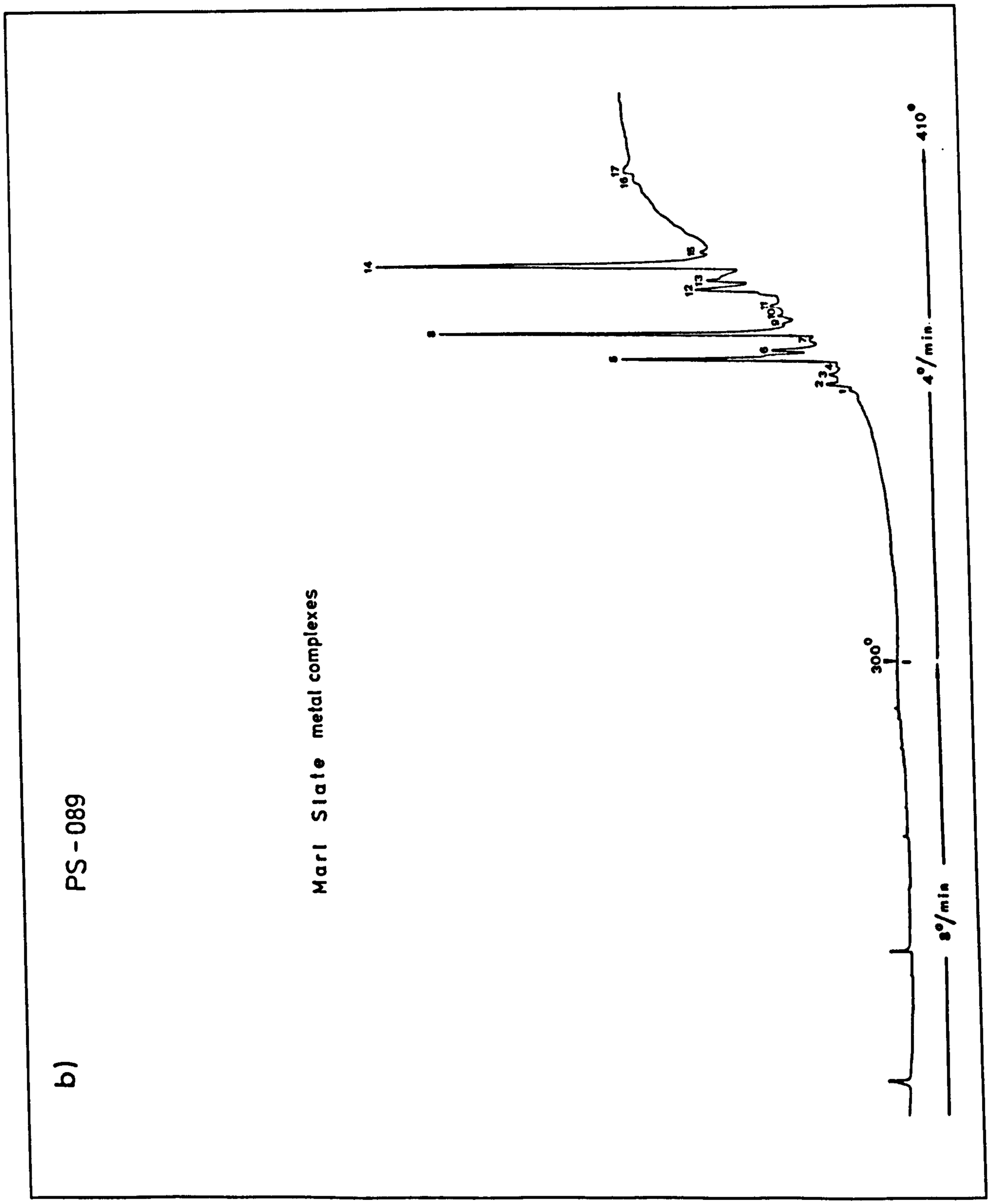


Fig.4b FID-chromatogram of a chloroform extract of preseparated Marl Slate oil shale analysed on a 20m x 0.3mm, PS-089 coated column.

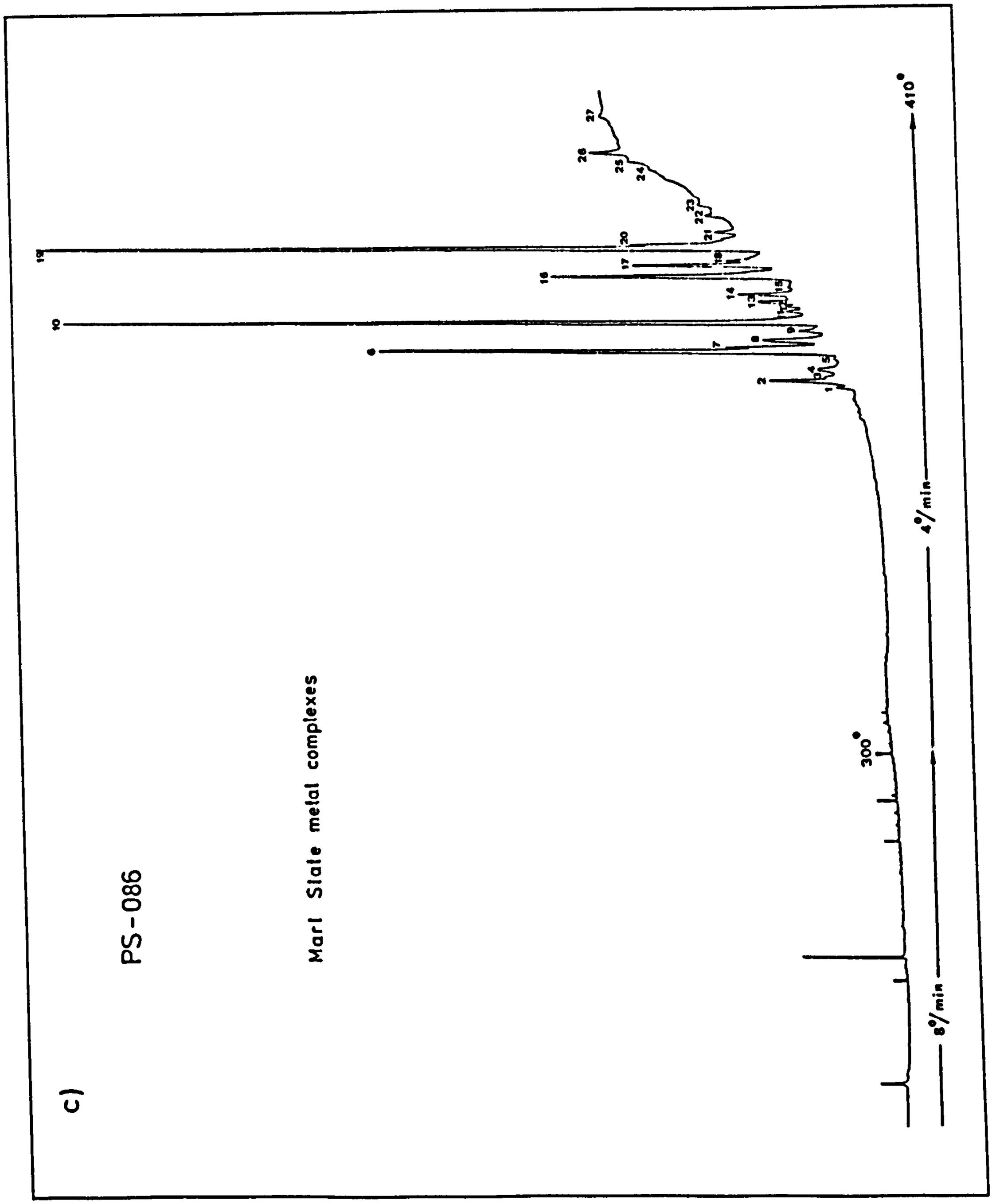


Fig.4c FID-chromatogram of a chloroform extract of preseparated Marl Slate oil shale analysed on a 20m x 0.3mm, PS-086 coated column.

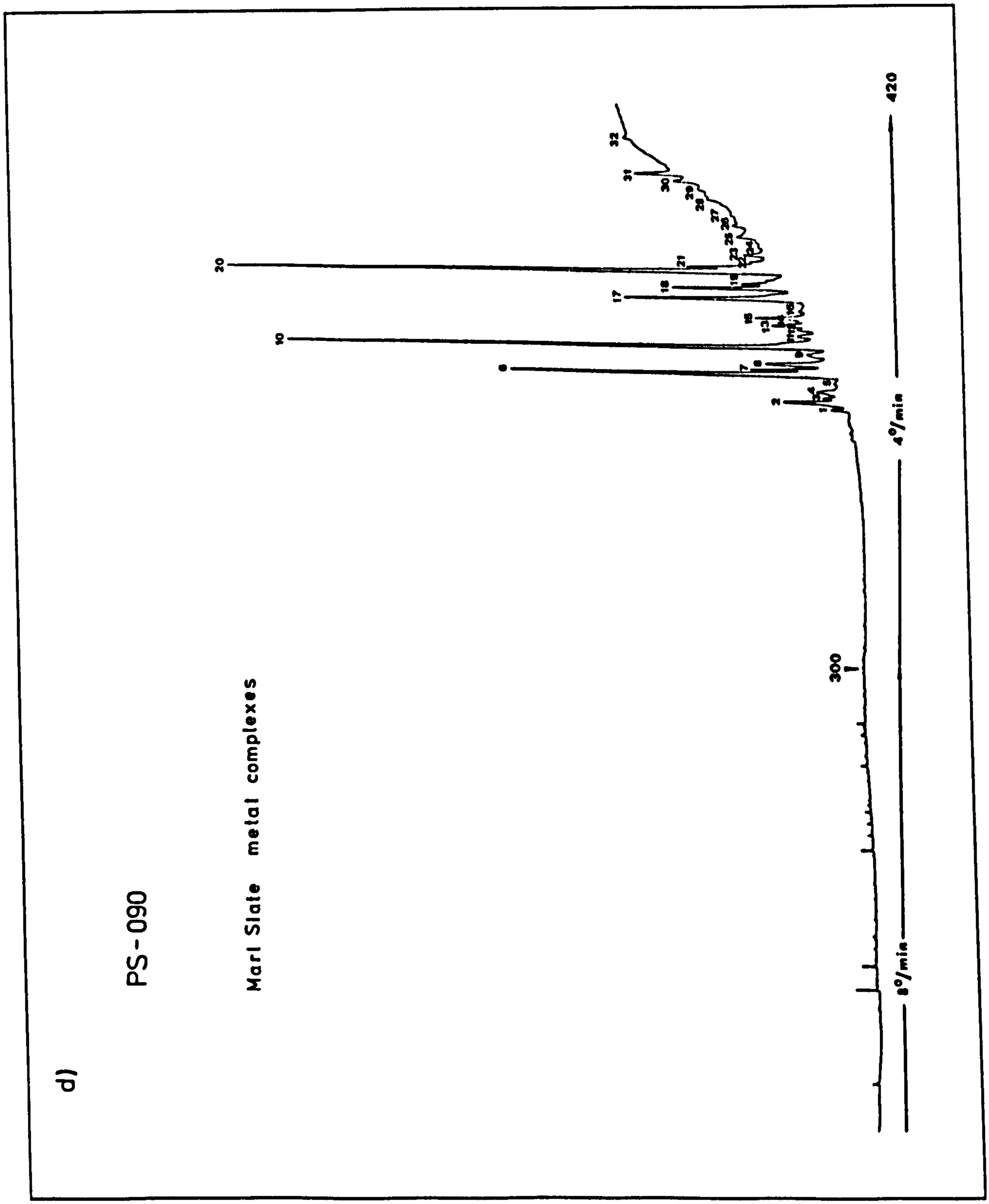


Fig.4d FID-chromatograms of a chloroform extract of preseparated Marl slate oil shale analysed on a 20m x 0.3mm, PS-090 coated column.

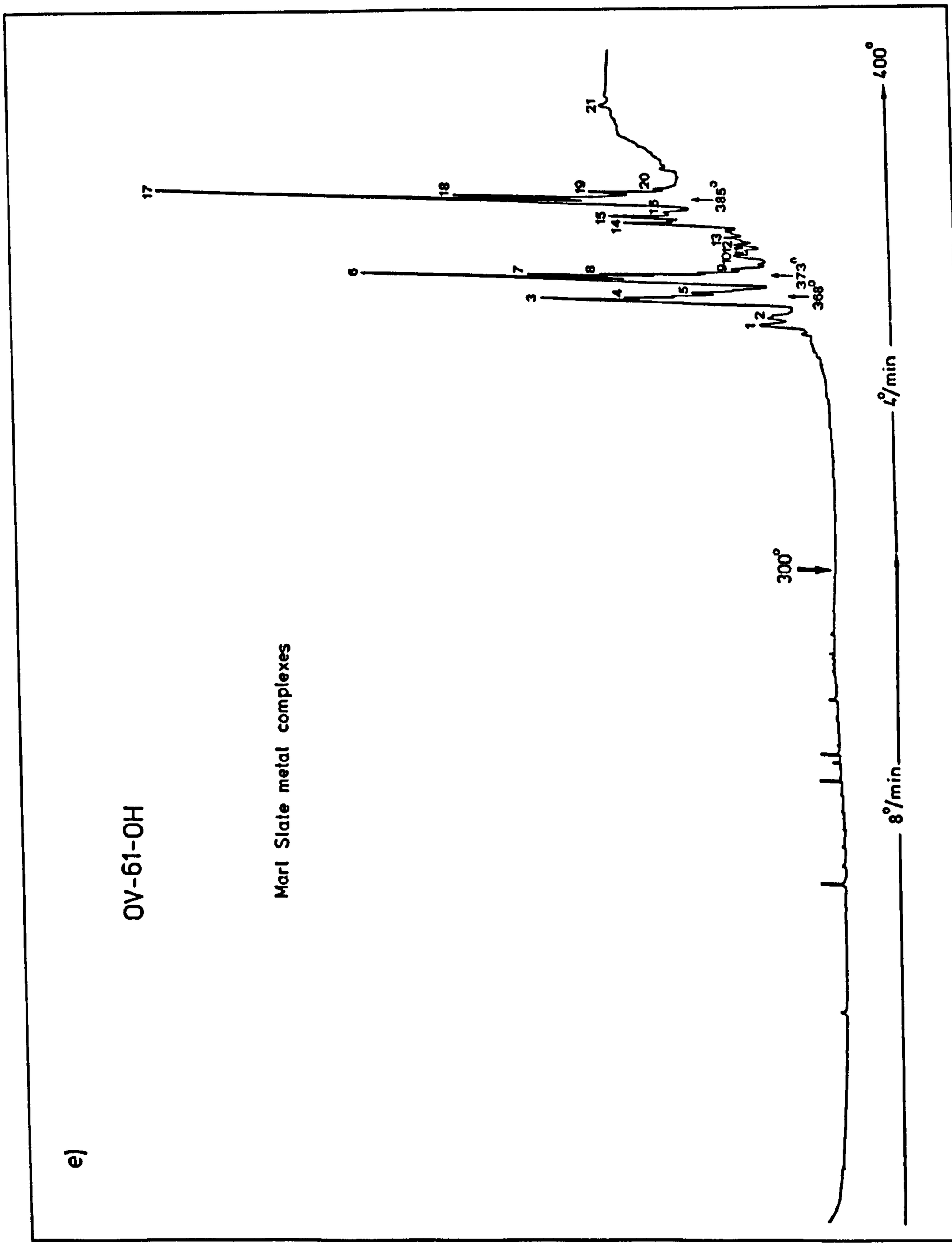


Fig.4e FID chromatogram of a chloroform extract of pre-separated Marl Slate oil shale analysed on a 20m x 0.3mm, OV-61-OH coated column.

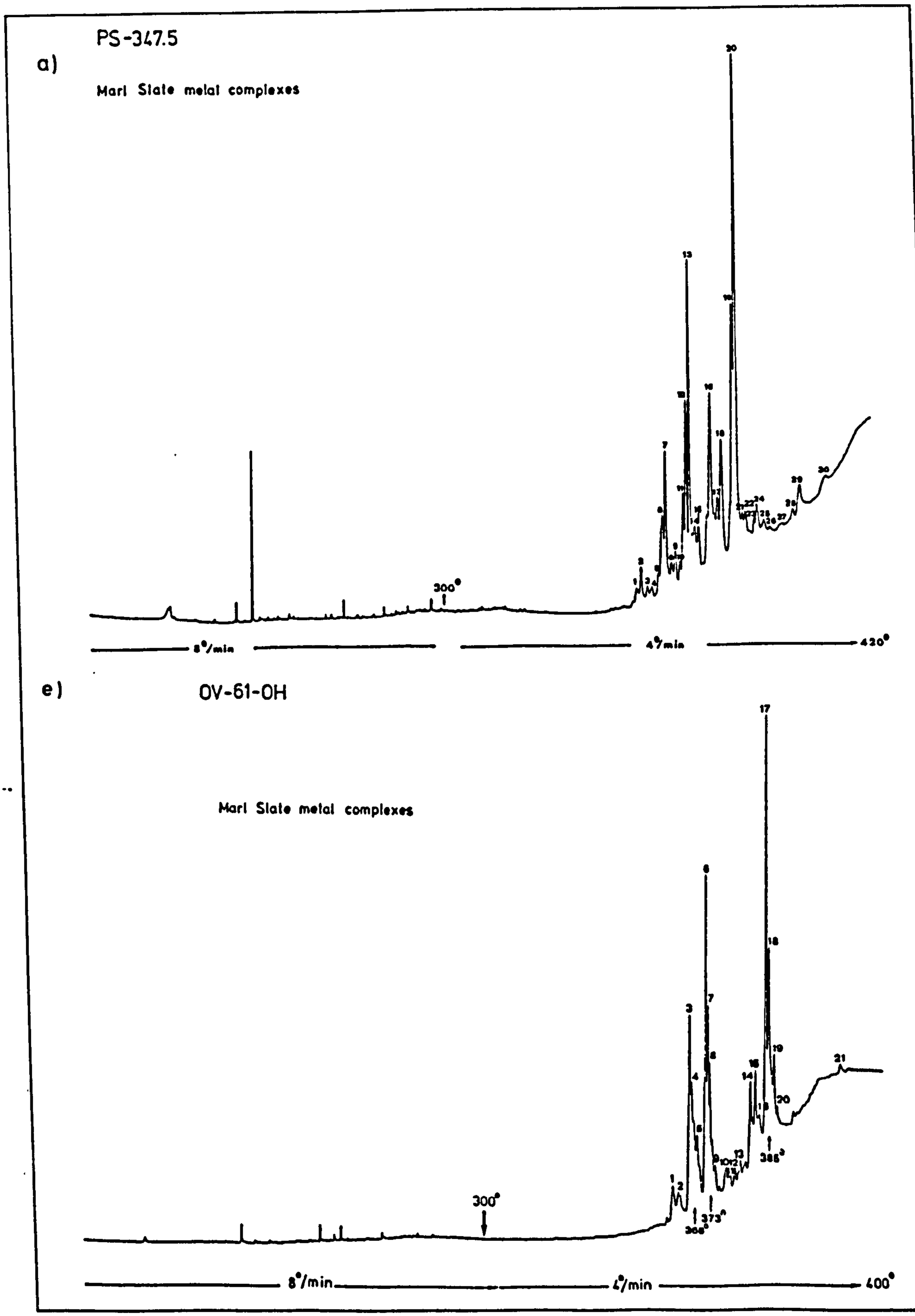


Fig.4f Comparison of porphyrin distribution of Marl Slate oil shale extract shown in Figs.4a and 4e. Note the inversion of the peak sequence.

I.3.2.3. Gas Chromatographic Separation of Metalloporphyrins with Different Central Atoms

Metalloporphyrins containing most metals of the Periodic System have been prepared and characterised (see Buchler, J.W. et al., 1975 and references cited therein), and the majority of tetrapyrrole pigments with physiological functions occur as metal complexes, but fortunately the number of chelating metals in metalloporphyrins present in organic matter of sedimentary origin is limited. Beside the widespread vanadium IV- and nickel II-complexes (Baker, E.W. et al., 1967, Maxwell, J.R. et al., 1980), there are manganese II- and iron III-porphyrins in immature coals (Bonnett, R. et al., 1981, 1983), gallium III-porphyrins in bituminous coals (Bonnett, R. et al., 1980,1984) and copper II-porphyrins in oceanic sediments and in humic coals (Palmer, S.E. and Baker, E.W., 1978; Louda, J.W. and Baker, E.W., 1981).

The different metals can be characterised after column liquid separation of the metalloporphyrins, by means of inductively-coupled plasma emission mass spectrometry (ICPMS). On the chromatogram below, showing an artificial mixture of octaethylporphyrin metallocomplexes with naturally occurring chelating atoms, it is demonstrated that different metalloporphyrins can be separated by capillary gas chromatography as well (Marriot, P.J. et al., 1982).

The subsequent characterisation of the metal atoms of some single GC peaks in various liquid chromatographic fractions of Julia Creek oil shale extract using both conventional GC/MS and electron impact ionisation (EI), as well as ICPMS is described in chapter V.

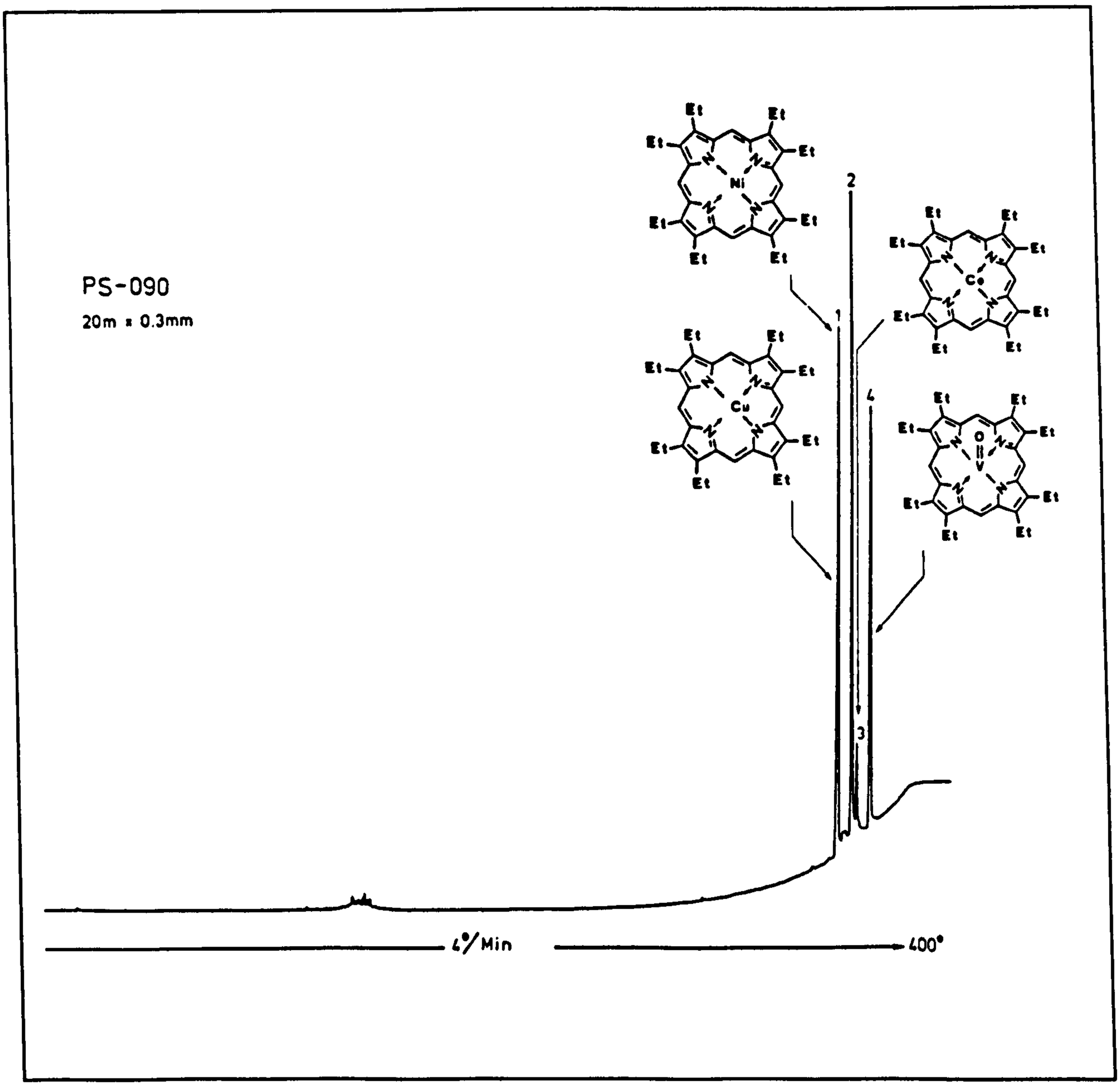


Fig.5 FID chromatogram of Cu(II)-, Ni(II)-, Co(II)- and VO(IV)-octaethyl-porpyrin. For experimental details see Section VII.1.3. From Blum and Eglinton 1989.

I.3.3. Analysis of Free Base Porphyrins

The GC analysis of the demetallated free base geoporphyrins implies similar working conditions as described for the analysis of metallocomplexes. The free bases prepared from some oil shale extracts elute at such low retention temperatures that they overlap

sometimes with the retention range of the hydrocarbons. In these cases the use of a nitrogen-specific, alkali flame ionisation detector (AFID) instead of a FID is recommended (see Section 1.3.7).

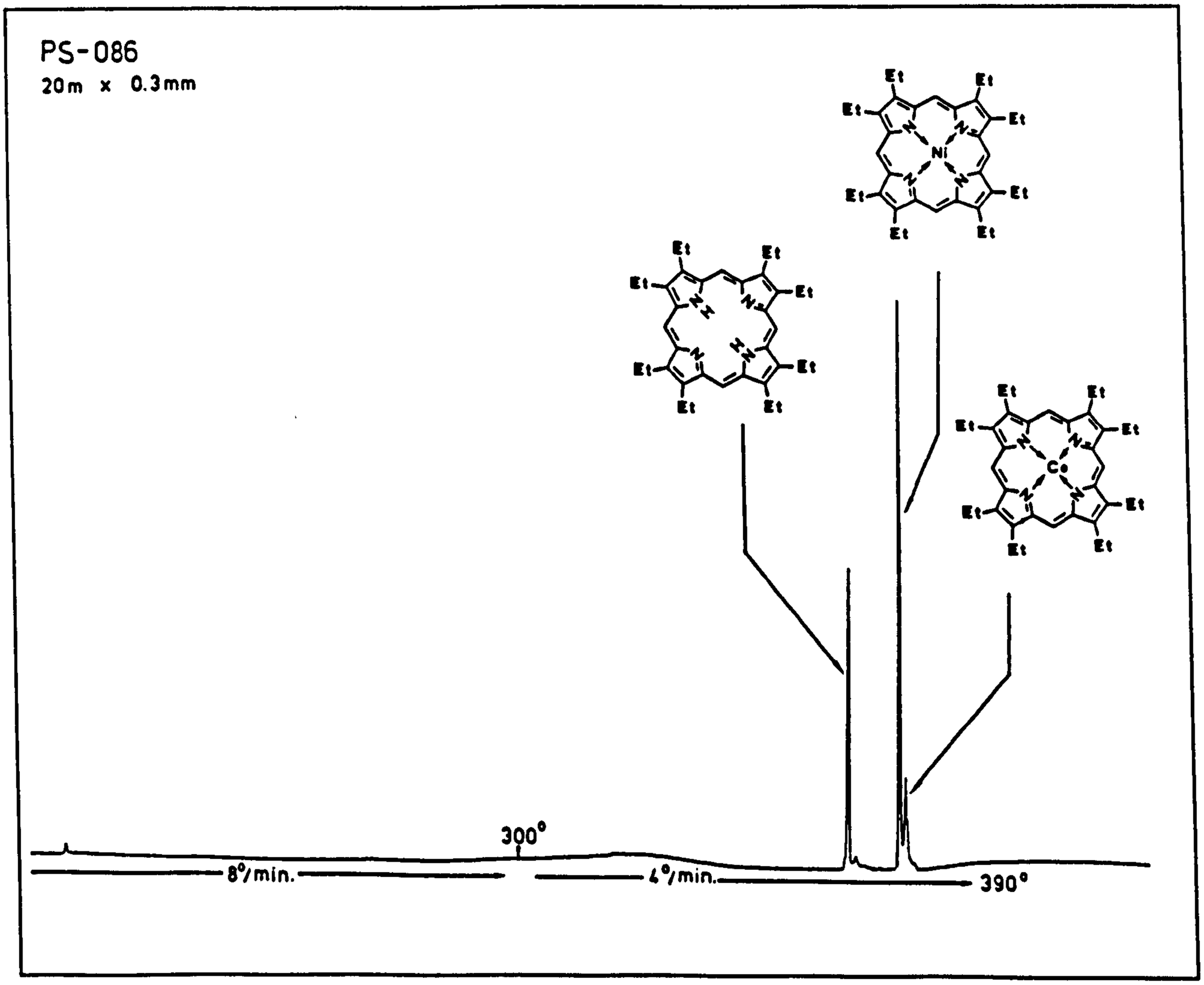


Fig.6 FID chromatogram of free-base, nickel- and cobalt octaethylporphyrin.

The retention temperatures of the free base of octaethylporphyrin are compared with the corresponding nickel- and cobalt complexes in Fig.6. As expected (see Section 1.2.), the free base appears at about 20°C lower column temperature and the maximum working temperature can therefore be reduced. Since for many demetallated oil shale extracts a temperature limit of 380°C is adequate, another high temperature stable but more polar and highly selective stationary phase OV-225-OH (50% methyl, 25% phenyl, 25% cyanopro-

pyl) can be used, especially for isomer separations (Blum, W. et al., 1988a).

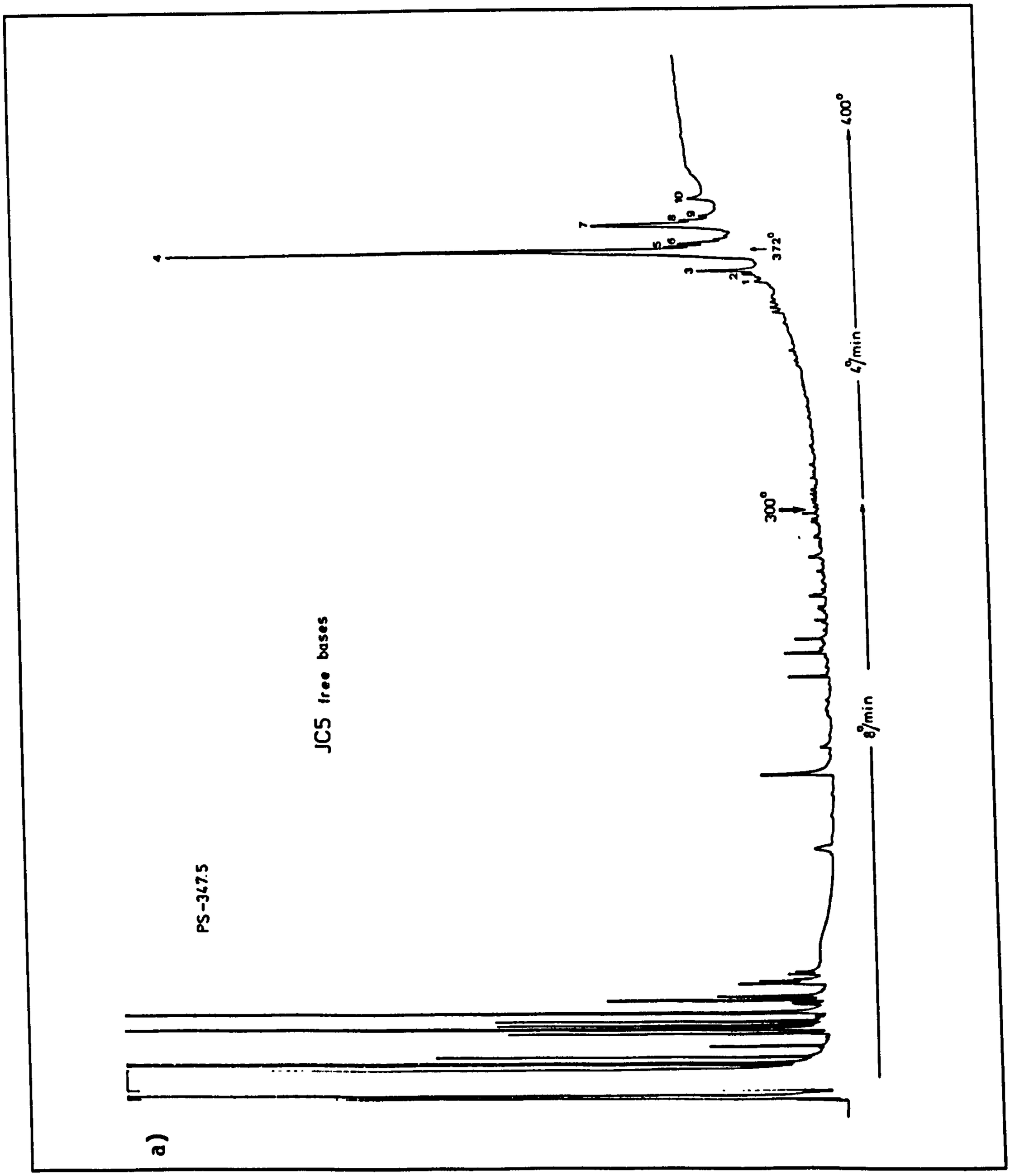


Fig.7a FID chromatogram of the demetallated fraction JC5 of Julia Creek oil shale on a 20 m x 0.3 mm PS-347.5 coated glass capillary column.

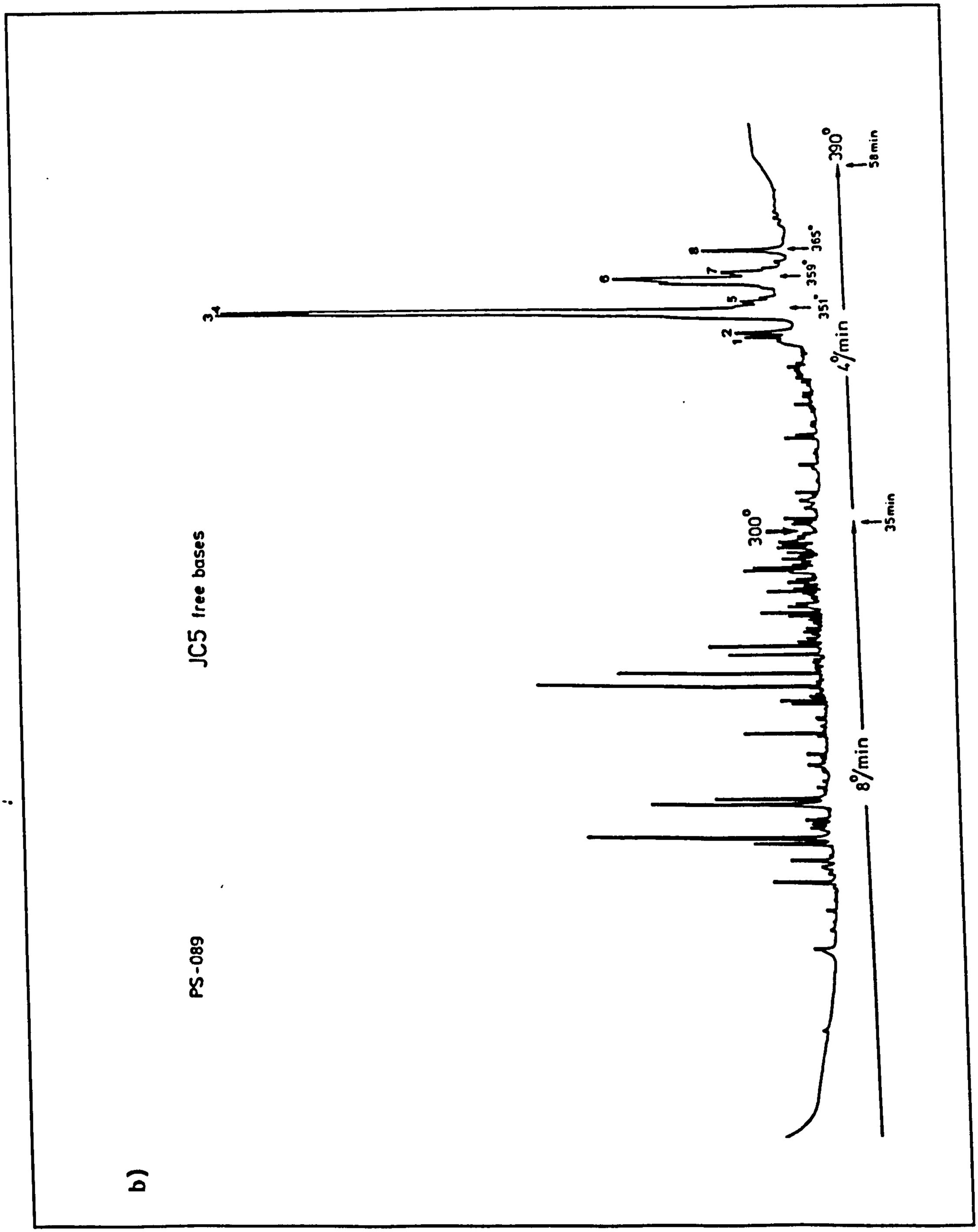


Fig.7b FID chromatogram of the demetallated fraction JC5 of Julia Creek oil shale, on a 20 m x 0.3 mm PS-089 coated glass capillary column.

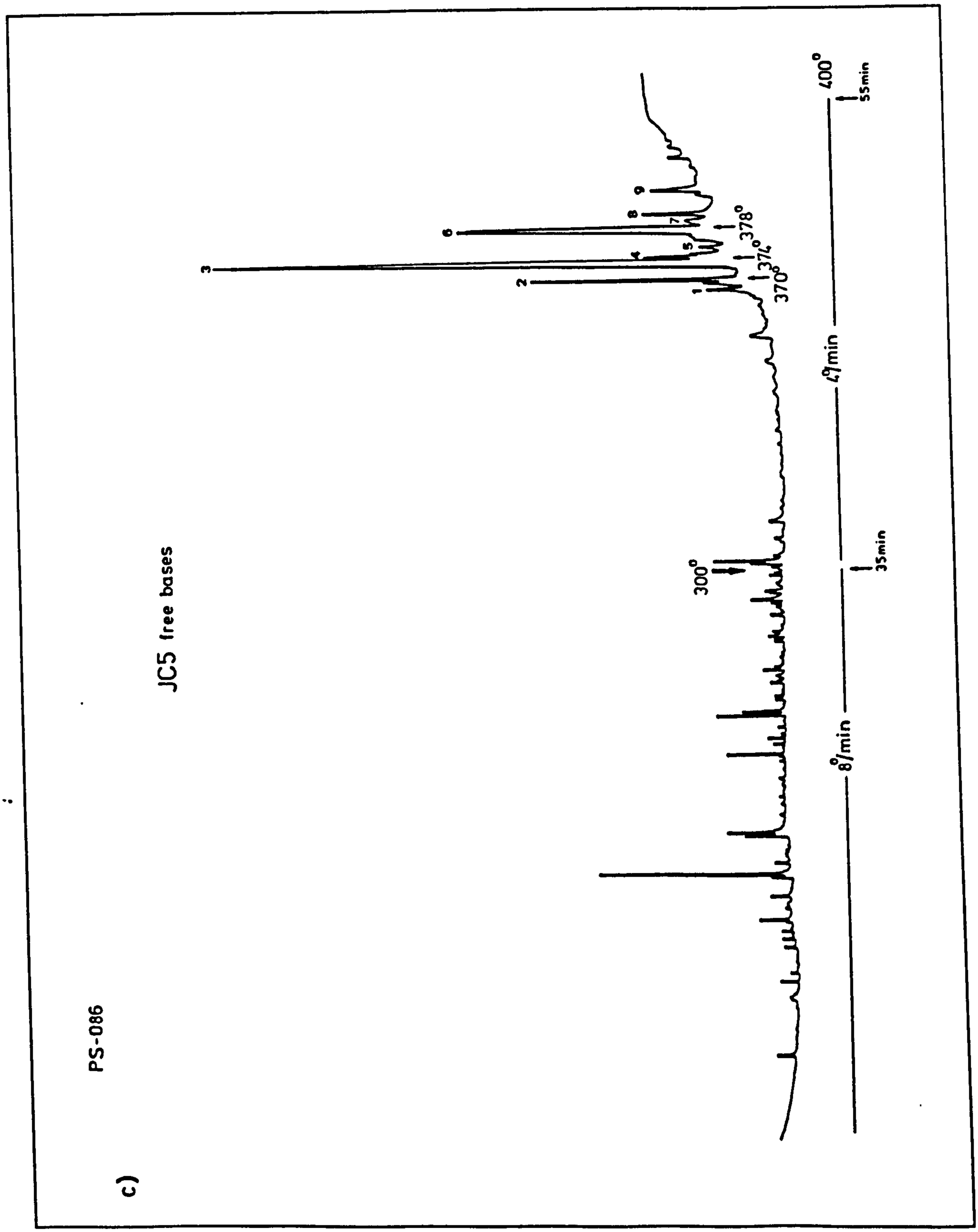


Fig.7c FID chromatogram of the demetallated fraction JC5 of Julia Creek oil shale, on a 20 m x 0.3 mm PS-086 coated glass capillary column.

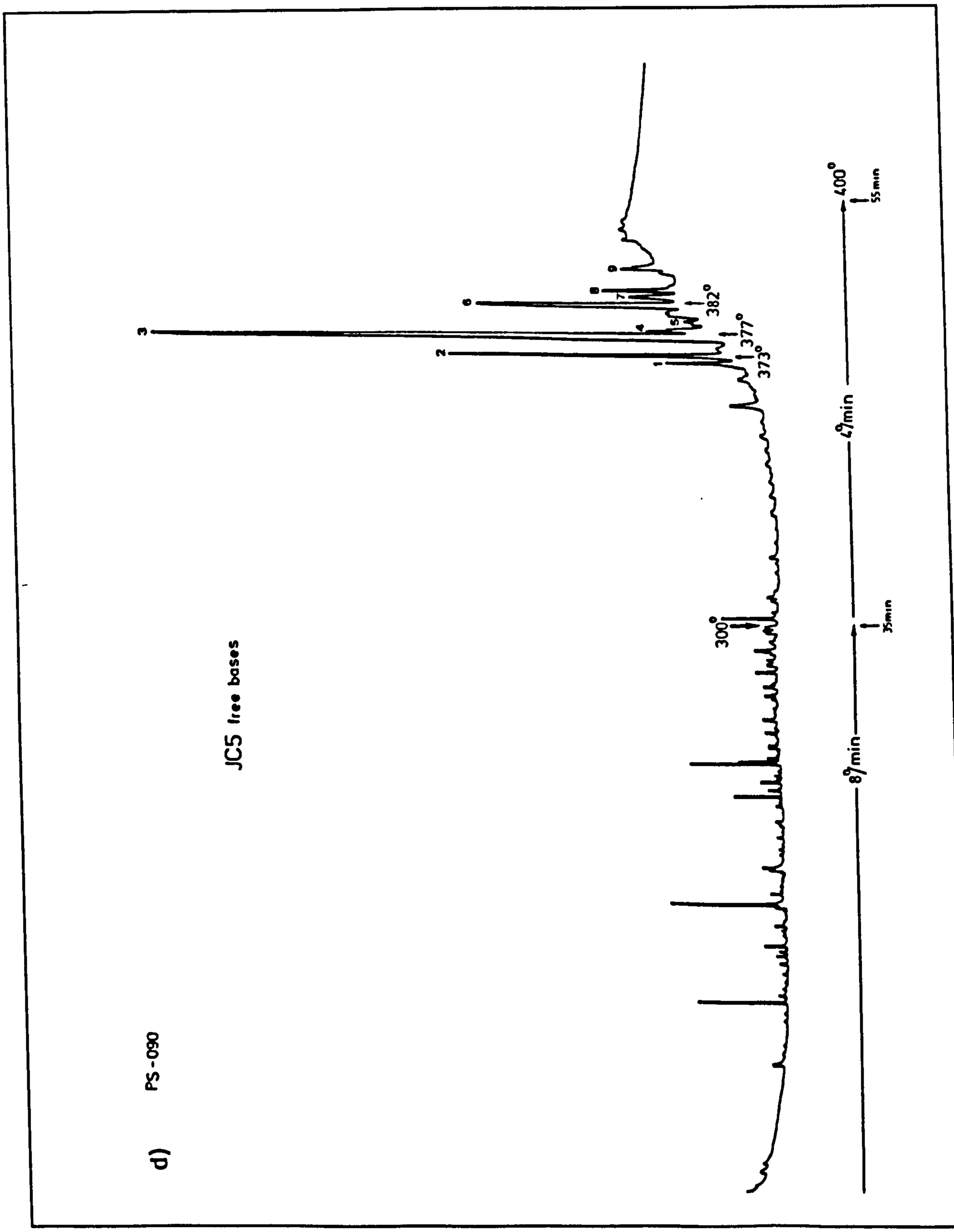


Fig.7d FID chromatogram of the demetallated fraction JC5 of Julia Creek oil shale, on a 20 m x 0.3 mm PS-090 coated glass capillary column

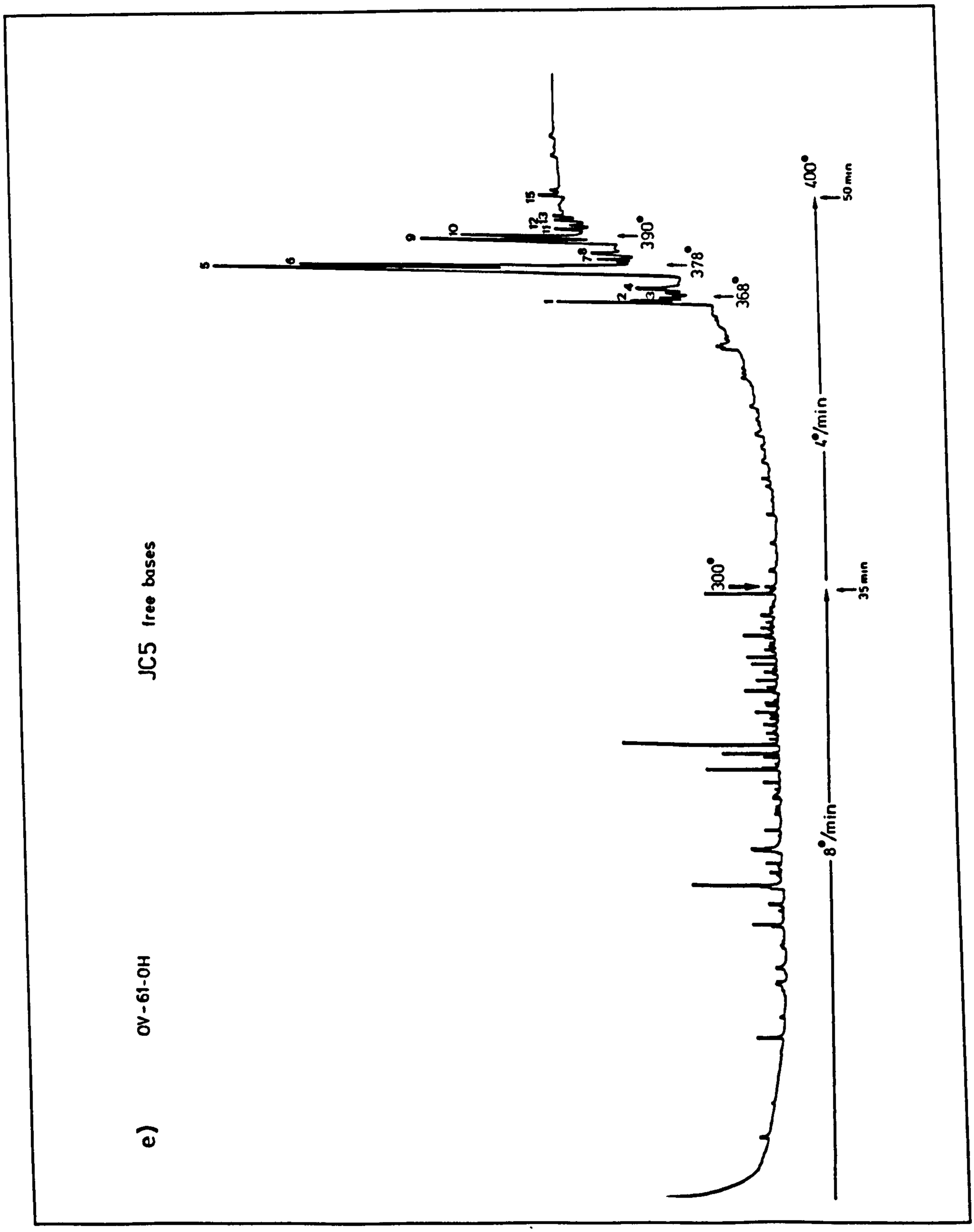


Fig.7e FID chromatograms of the demetallated fraction JC5 of Julia Creek oil shale, on a 20 m x 0.3 mm OV-61-OH coated glass capillary column.

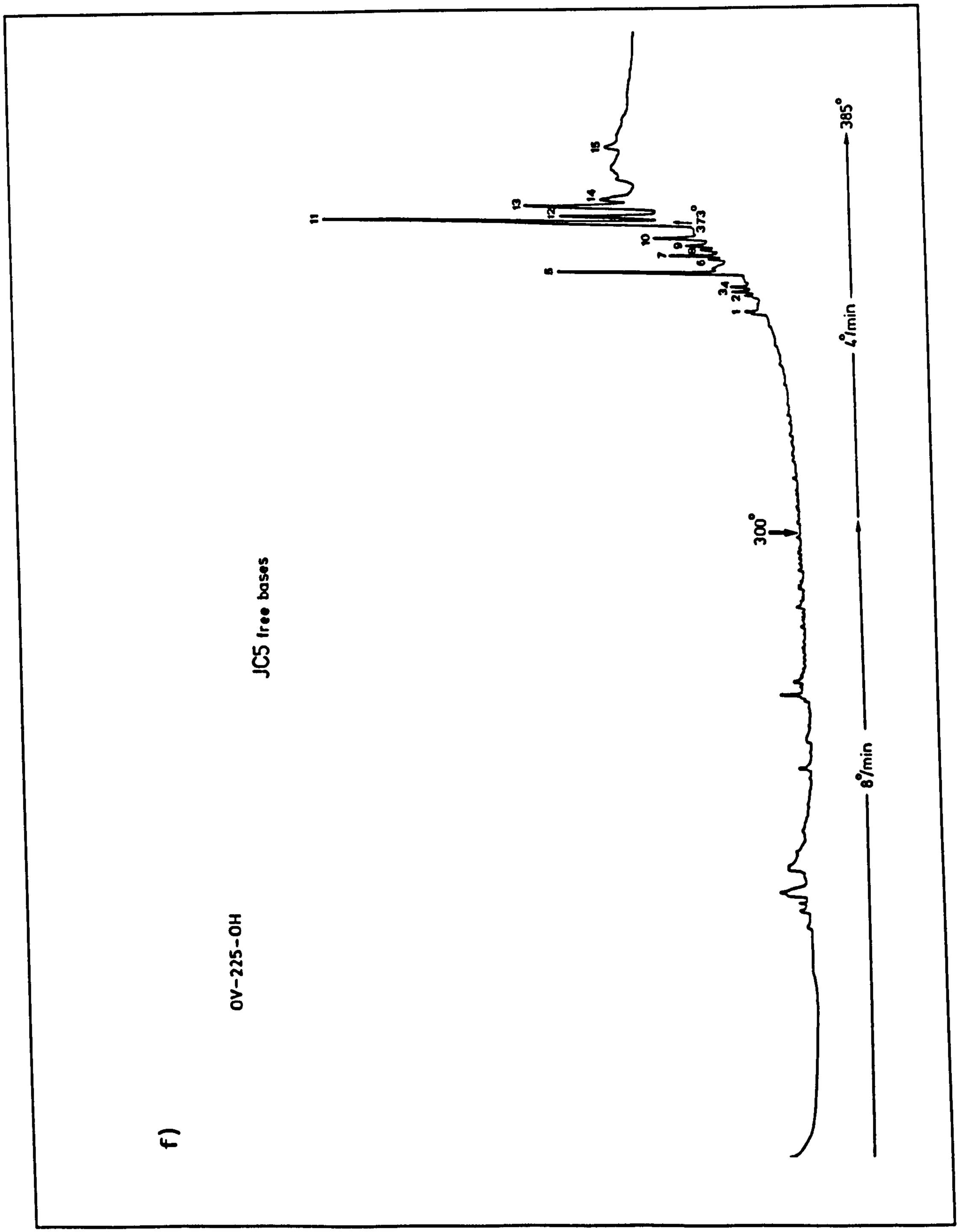


Fig.7f FID chromatogram of the demetallated fraction JC5 of Julia Creek oil shale, on a 15m x 0.3mm, OV-225-OH coated glass capillary column.

Owing to the different nature of their interactions, more polar stationary phases alter the retention behaviour of porphyrins and improve the isomer separation (see Figs. 7e and 7f).
The higher separation efficiency obtained for the free bases parallels the results obtained by high pressure liquid chromatography over silica (Barwise, A.J.G. et al., 1986).

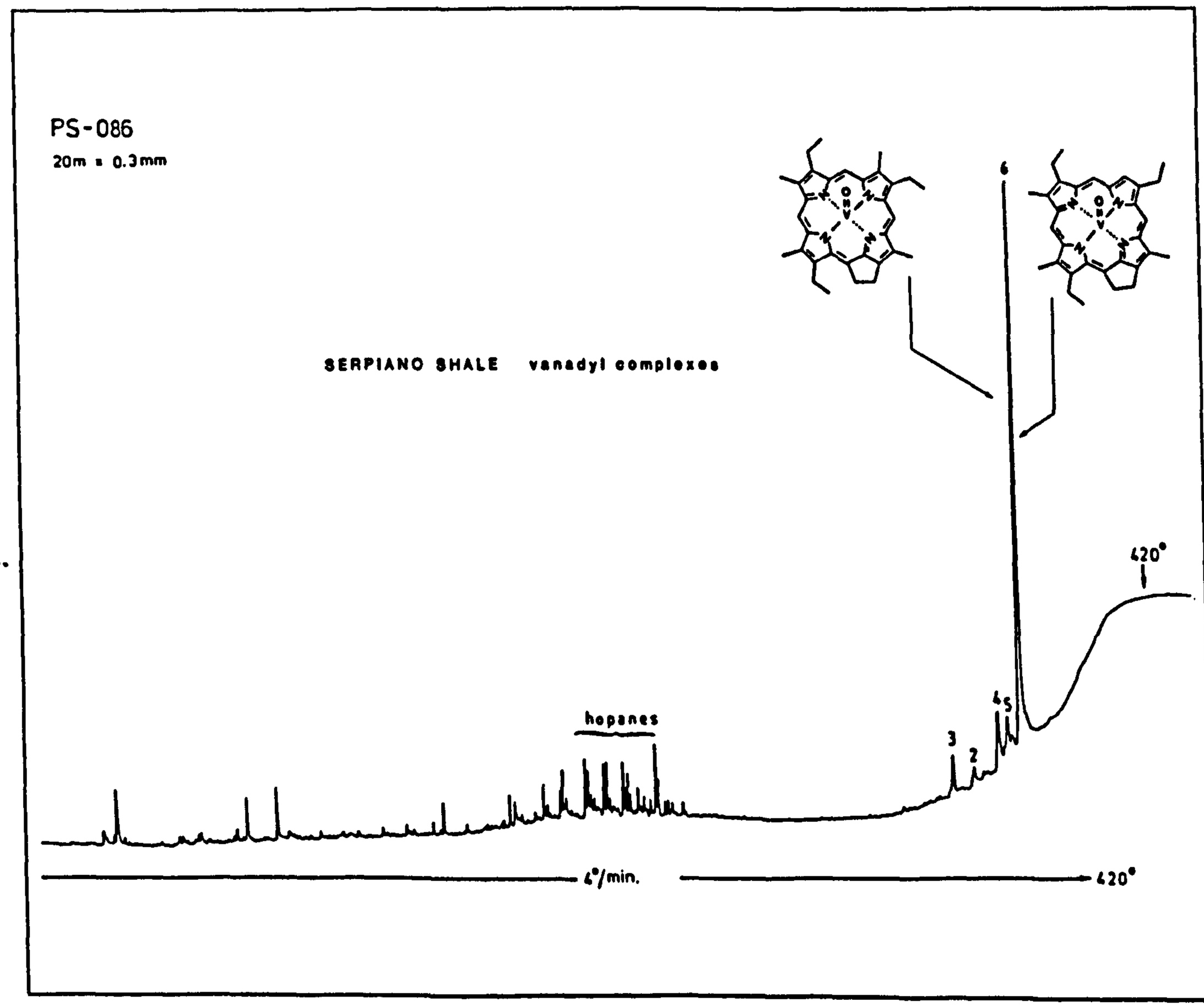


Fig.8a FID trace of metallated Serpiano oil shale extract. From Blum et al. 1988a. The two porphyrin homologue structures are assigned from HPLC separations (Chicarelli, I., 1987) and GC/MS measurments.

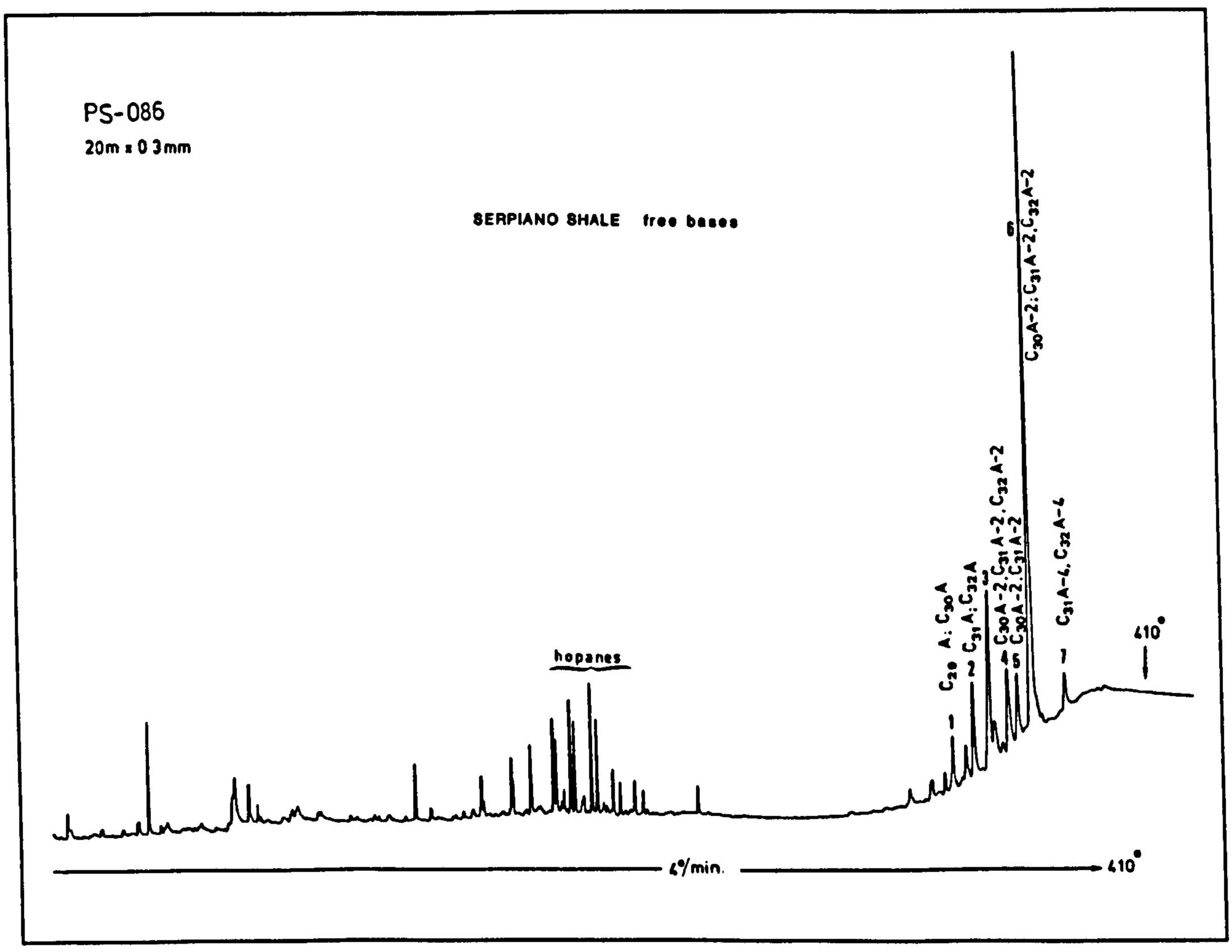


Fig.8b FID trace of a demetallated trichloromethane extract of Serpiano oil shale. For both chromatograms the same column and the same operation conditions are used. From Blum et al., 1988a.

I.3.4. Selection of the Carrier Gas and Constant Flow Regulation

The selection of the carrier gas for high temperature gas chromatography depends above all on a consideration of its viscosity. Owing to the increase of carrier gas viscosity in parallel to an increase of column temperature, a pressure- and flow drop off is obtained resulting in a decline of separation efficiency, particularly at very high temperatures. Since hydrogen has about

half the viscosity of the commonly preferred helium or nitrogen, its use not only shortens the analysis time, but also extends the application range at a higher separation efficiency. Therefore, it is essential to use hydrogen as carrier gas in the analysis of free base- and metalloporhyrins and it was exclusively used in this study.

An increase in column temperature would lead to a decrease in hydrogen flow if just the pressure was held constant. Consequently a flow drop would result when a temperature program is run. In order to avoid this, constant flow regulation was applied in this study. A commercially available device (see Section VII.2.1.) gradually increased the hydrogen pressure in parallel with the increase in temperature, thus keeping the gas flow constant over the whole temperature range.

The separation efficiency is expressed in (temperature dependent) TZ values (TZ=Trennzahl, separation number, Kaiser, R., 1962) rather than temperature independent plate numbers. TZ values are simply calculated according to:

$$TZ = \frac{t_{Cn} - tC_{n-1}}{w_{Cn} + w_{C_{n-1}}} - 1$$

Whereby t_{Cn} and t_{Cn-1} are the corrected retention times of the neighbouring pair of homologues C_n and C_{n-1}, respectively, and W_{Cn} and W_{Cn-1} are there peak widths. The TZ gives the number of base line separated peaks, fitting between this neighbouring homologues. In practice TZ values provide more reliable measurements of separation efficiency than plate numbers (Grob, K., 1981).

Figs.9 and 10 show a series of alkane homologues measured in constant pressure and constant flow mode, respectively. They clearly demonstrate the differences between both modes, especially the difference with respect to the extended working range.

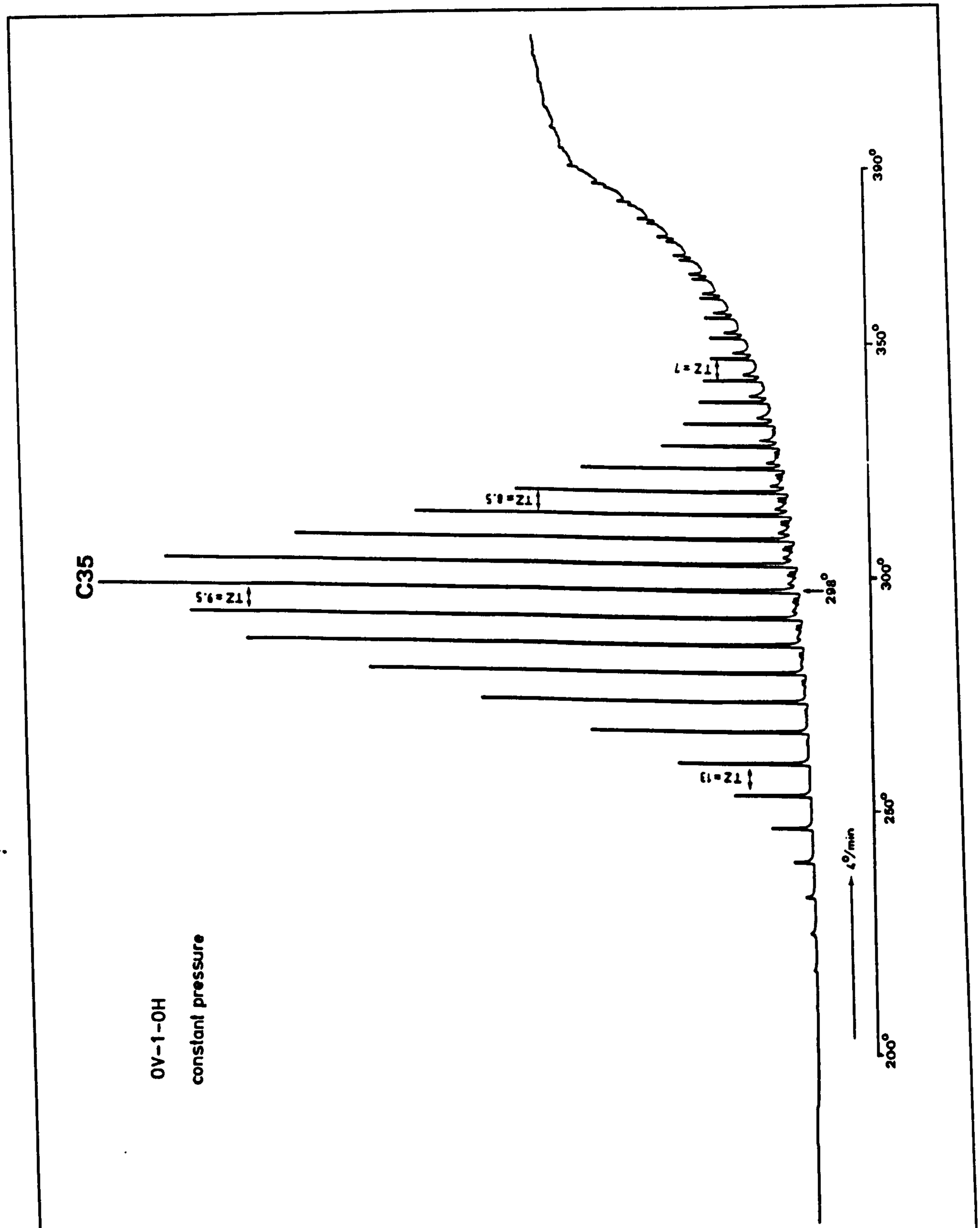


Fig.9 FID chromatogram of homologue alkanes in constant pressure mode.
Column: OV-1-OH, 18m x 0.3mm; carrier gas: hydrogen

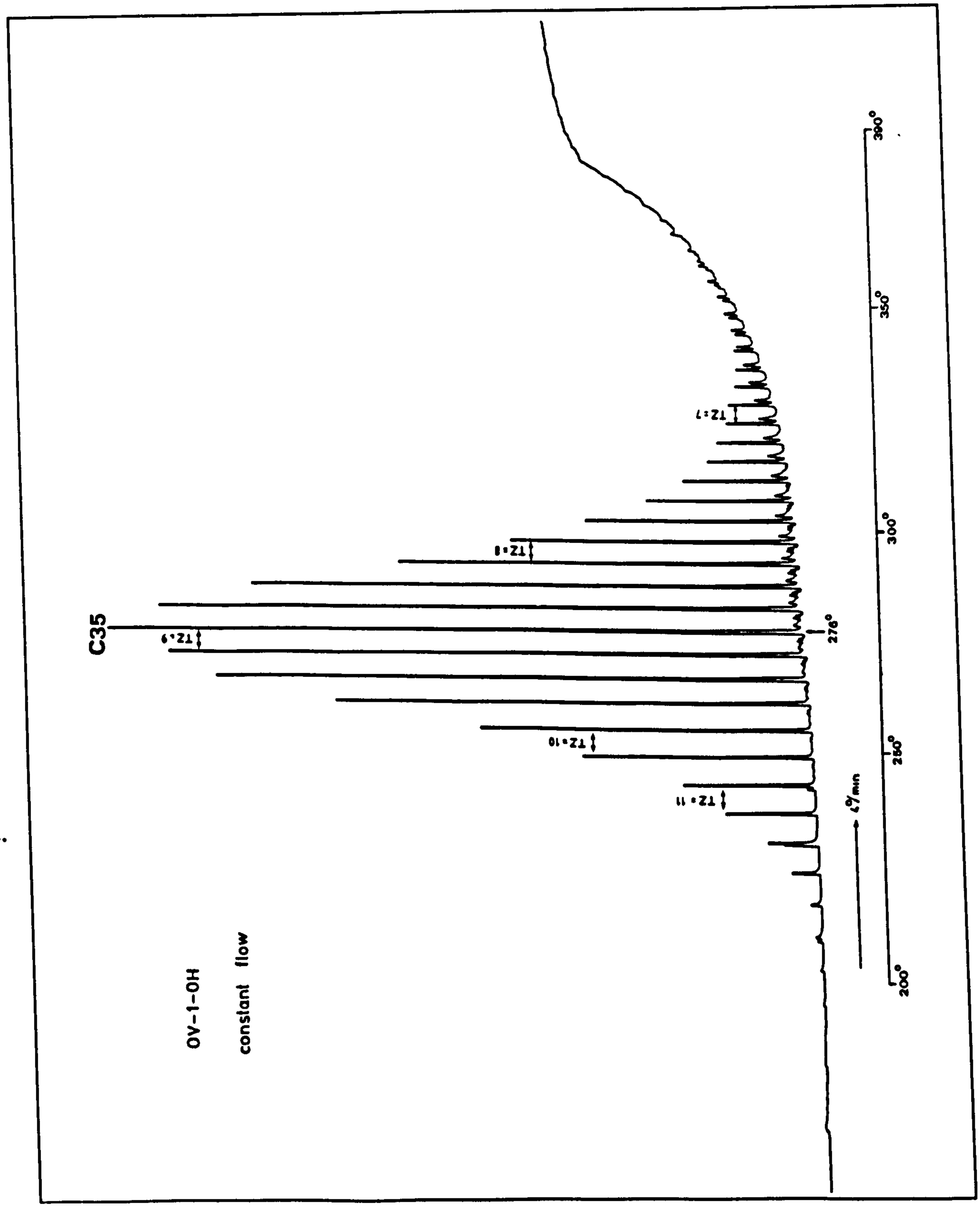


Fig.10 FID chromatogram of homologue alkanes in constant flow mode.
Column: OV-1-OH, 18m x 0.3mm; carrier gas: hydrogen

The two chromatograms reveal (i), that the TZ values are inversely proportional to the column temperature (ii), that the application range in constant flow can be extended by about 20°C, and (iii), that a higher separation efficiency is obtained in constant pressure mode in the temperature range around 200°. At temperatures above 350°C both modes show nearly the same TZ values.

For the direct GC/MS analysis of free base- and metalloporphyrins, the extension of the working range is of principle importance. It helps to lower the "bleeding" rate of a column, which, particularly with medium polarity coatings, can be a limiting factor and therefore increases the durability. Thus, the applications shown in Chapter V exclusively employed constant flow mode.

I.3.5. The Use of Specific Atmospheric Detectors (AFID)

Besides the widely used flame ionisation detector (FID) the thermionic nitrogen/phosphorus selective alkali flame ionisation detector (AFID) can also be used in high temperature capillary gas chromatography up to 450°C. The basic design of the AFID is identical to the FID except that an alkali metal salt source is placed between the burner tip and the collector. The detection is based on an increase in current through a hydrogen flame, which has been seeded with alkali salt, when organonitrogen or -phosphorus compounds are introduced. The current increase is caused by CN or NO_2 radicals which are believed to be formed during the thermal decomposition of the organonitrogen compounds (van de Weijer, P. et al. 1988). Like the FID, the AFID is a destructive and mass flow-rate dependent detector.

Since the AFID is highly selective and sensitive to compounds containing nitrogen it has some clear advantages over the nonselective FID, particularly in the case where the retention range of the free-base extract from a crude mixture(or its derivatives, respectively) overlaps with an unavoidable cluster of hydrocarbon peaks. The AFID cannot be used in order to detect metalloporphyrins. For unknown reasons, many of the chelated metalloporphyrins gave a negative detector response. The use of the AFID is demonstrated with a demetallated Serpiano oil shale extract in Fig.11.

Owing to the fact that the sodium loss is not controlled by evaporation but by a sodium/hydrogen exchange, the AFID response depends on the concentration of hydrogen in the gaseous boundary layer of the alkali metal bead. Accurate control of the hydrogen flow of the detector, and (if hydrogen is used as a mobile phase) also of the carrier gas, is therefore important. A change in hydrogen flow rate of only 0.05 % leads to a change in the ionisation current of 1 % (Hartman, C.H., 1966). Consequently, a highly stable flow rate of the detector supply and a highly constant carrier gas flow is required. As shown from the base line in Fig.11, this is obtained with the constant pressure/constant flow regulator generally recommended for high temperature capillary gas chromatography (see Section 1.3.4.).

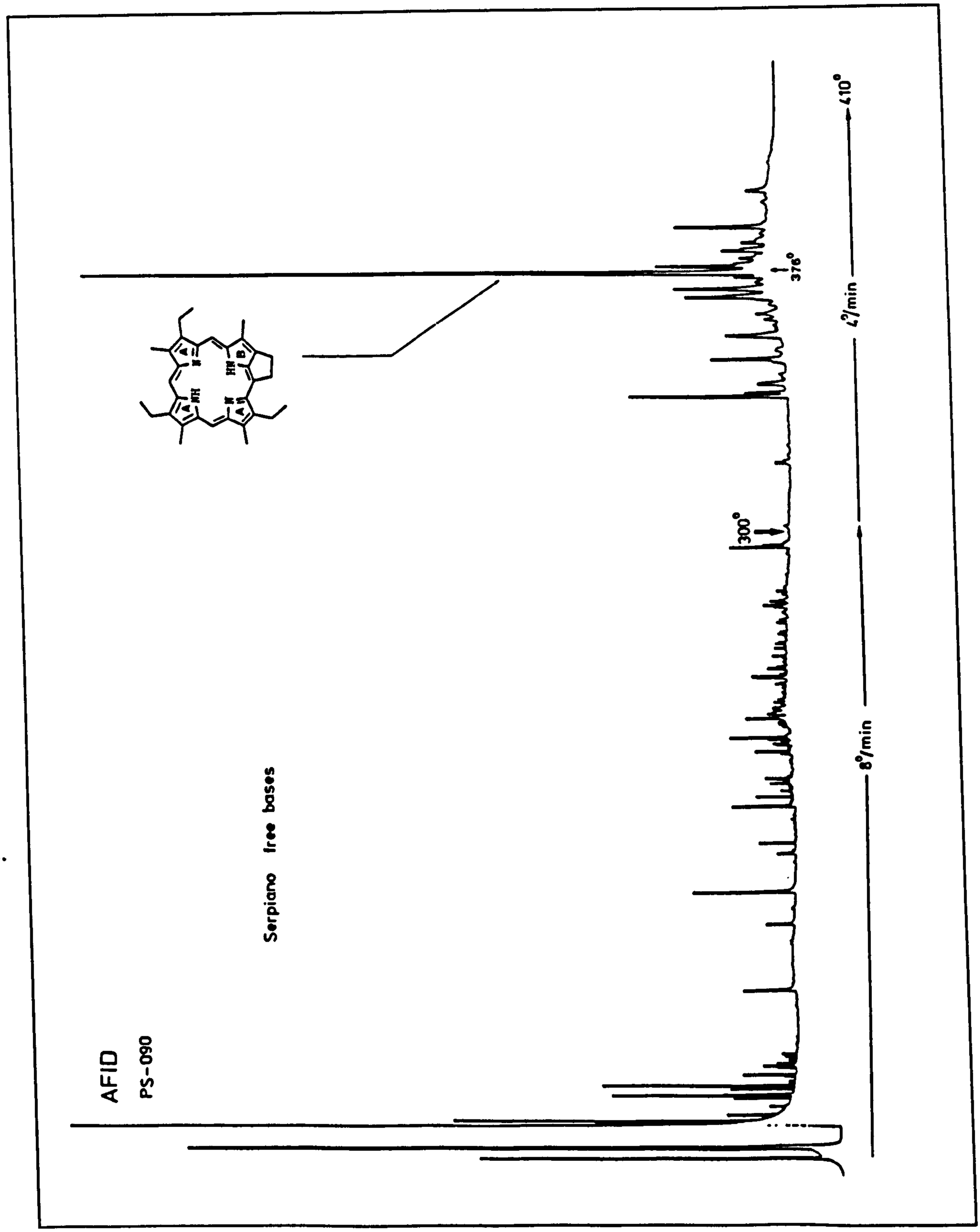


Fig.11 AFID chromatogram of a pre-separated and demetallated Serpiano oil shale extract. For the corresponding FID chromatogram see Fig.8b.

II. SUPERCRITICAL FLUID CHROMATOGRAPHY OF PORPHYRINS

The first successful application of supercritical fluid chromatography was the separation of the nickel(II) complexes of aetioporphyrin II (10) and mesoporphyrin IX DME (11) by Klesper et al. (Klesper, E. et al., 1962). The authors used a packed column and supercritical CF_2Cl_2 or CHF_2Cl, respectively, as mobile phases. Some years later Karayannis et al. (Karayannis, N.M. et al.,1968) described the supercritical fluid chromatographic separation of some porphyrins from biological sources (e.g. mesoporphyrin IX dimethylester (11) and deoxophylloerythrin methyl ester (12) etc. but also of aetioporphyrin II chelates of a range of metals (Karayannis et al. 1970). Unfortunately, the relatively low separation efficiency of SFC, particularly on packed columns, makes the method ineffective for separation of complex geoporphyrin mixtures.

Attempts to elute octaethylporphyrin with supercritical pentane from open tubular columns were reported by Jackson (Jackson, W.P., 1985). Fields et al. reported that the use of carbon dioxide failed in the same application but that a mixture of 8.9 mole of isopropanol/carbon dioxide as a mobile phase eluted vanadyl octaethylporphyrin from a 10m x 0.05mm capillary column (Fields, S.M. et al. 1988). This author also compared the selectivity of different methyl phenylpolysiloxane stationary phases with the result that the best separation has been achieved on a phase containing 30% of biphenyl functions.

The first mixture analysis (geoporphyrins from Meride Shale) was performed by Fields using isopropanol/CO_2 as mobile phase (Fields, S.M., 1987). The respective capillary supercritical fluid chromatogram is shown in Figure 12.

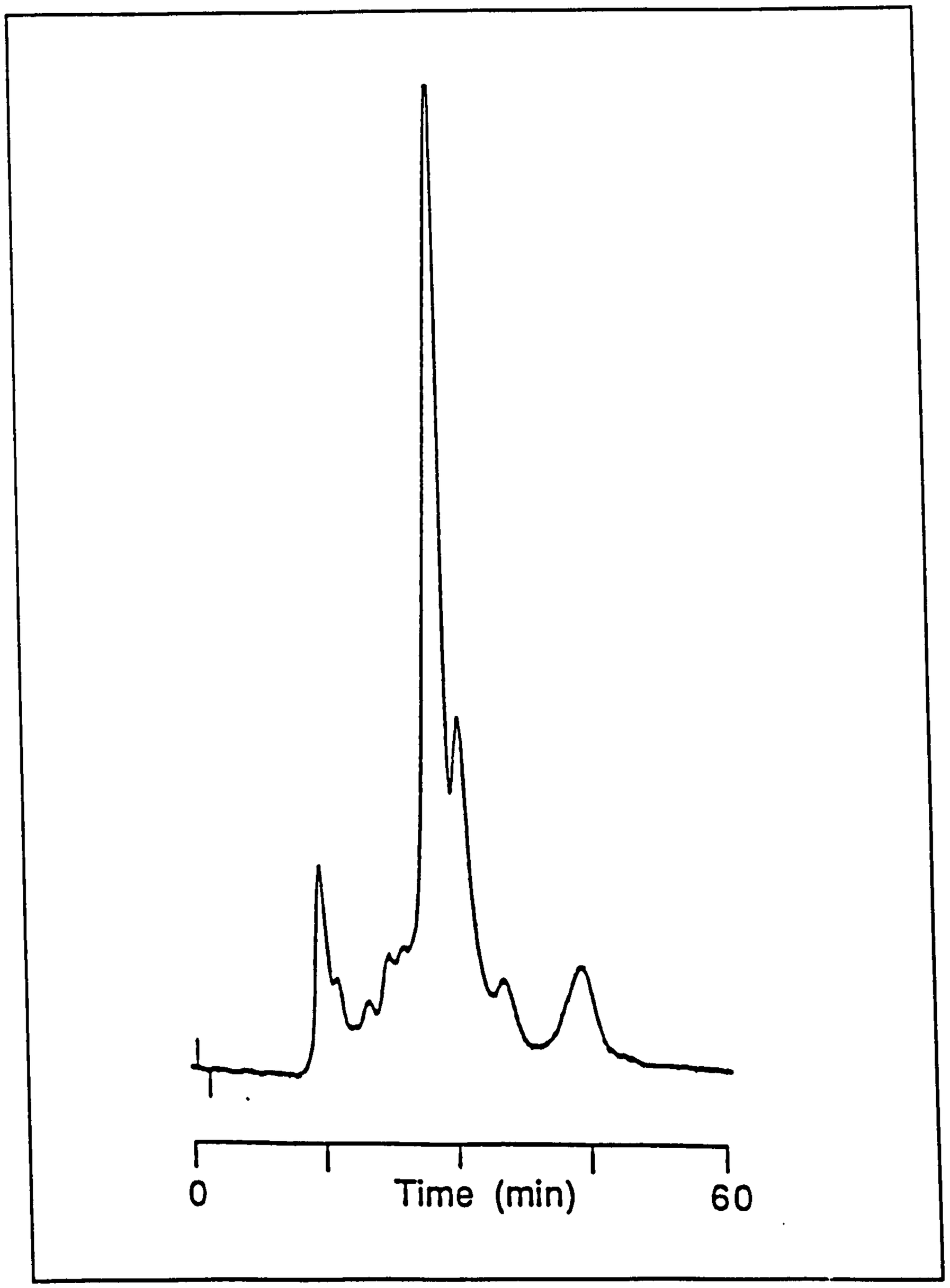


Fig.12 UV-Supercritical fluid chromatogram of metalloporphyrin extract of Meride shale. 6m x 0.05mm fused silica column coated with 30% biphenylmethylpolysiloxane 0.25um. Mobile phase: 8.9 mole% isopropanol/ CO_2, 220 atm, 120°. UV-detection at 406nm.
From Fields, S.M., 1987.

III. SIMULTANEOUS COUPLING OF HIGH TEMPERATURE GLASS CAPILLARY GAS CHROMATOGRAPHY AND OF CAPILLARY SUPERCRITICAL FLUID CHROMATOGRAPHY TO A MASS SPECTROMETER

III.1. The Coupling of High Temperature Glass Capillary Columns to a Mass Spectrometer

The main criteria for the successful coupling of a capillary column to a mass spectrometer are:

- The introduction of the total column effluent into the ion source should not influence the operation and performance spectrometer.

- The chromatographic resolution recorded by means of total ion current detection should be the same as that observed with atmospheric pressure detectors.

- The performance and sensitivity of the mass spectrometer should not be affected by changes of the column dimensions, the nature of the carrier gas and the GC parameters.

- The coupling of the capillary column (even of glass) should be easily and quickly accomplished.

When high temperature glass capillary columns are used none of the commercially available interface systems fulfils these requirements. One problem encountered in interfacing capillary columns to a mass spectrometer is the free selection of the carrier gas. Since hydrogen has about half the viscosity of the commonly preferred helium or nitrogen its use not only shortens

the analysis time, but also extends the chromatographic application range at higher separation efficiency. Unfortunately, apart from the fact that most of the present GC/MS systems suffer from insufficient pumping capacity (leak rates <5ml He), they are preferentially equipped with turbomolecular pumps, which are notoriously weak if the mass spectrometer has to be kept evacuated against a continuous flow of hydrogen. Furthermore, the interface temperature of about 400°C (close to the maximum working temperature of the column) should be kept as constant as possible along the interface line up to the very end in order to avoid "cold spots". The extension of the exit of flexible fused-silica columns into the ionisation chamber, as is common practice, will create serious background problems, produced by volatile ingredients of the polyimide outside coating. The background increases more dramatically if in chemical ionisation mode the reagent gas is introduced parallel to the column effluent.

In analytical practice, chemical ionisation (CI) is in many cases the better choice than electron impact ionisation (EI) for the investigation of large molecules. Consequently, a high temperature GC/MS interface should allow both modes of ionisation without any limitations.

Since the first reports on capillary GC/CI-mass spectrometry by means of a dual gas system (Blum, W. and Richter, W.J., 1973; 1974) (in contrast to earlier mono gas arrangements these systems use separate carrier and reagent gas inlets), the parallel introduction of both CI reagent and carrier gas has proven to be the most efficient coupling arrangement. Later it was optimised to the coaxial dual gas interface (Blum, W. and Richter, W.J., 1975), equipped with a glass transfer line and extended by a heated vaporiser for polar liquid reagent gases (Blum, W. and Richter, W.J, 1977).

The second generation of a glass coaxial dual gas system shown in Fig.13 not only takes into account the requirements of EI and CI, but is also easily adaptable to any GC system.

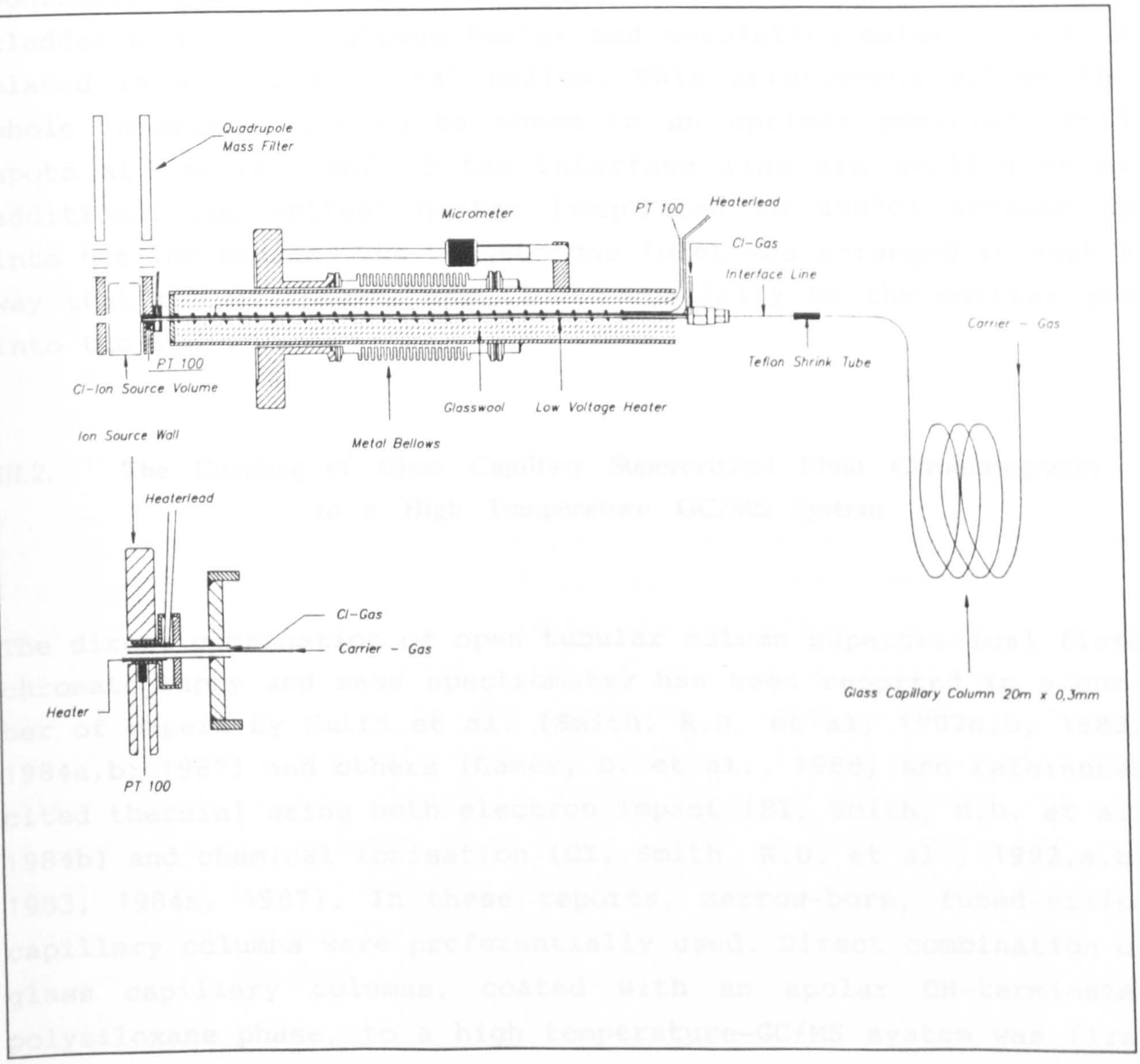


Fig.13 Schematic illustration of the high temperature glass capillary interface linked to the quadrupole mass spectrometer. The enlarged representation below shows the entrance into the ionisation chamber with additional electrical heater and the CI reagent gas entrance coaxial to the interface capillary.

A pretreated glass interface line (for the pretreatment see Section VII.2.3.1.) with an i.d. of 0.2mm sits in a temperature controlled oven. The oven consists of a stainless steel capillary cladded with a low voltage heater and insulating material and is placed in a flexible metal bellow. This arrangement allows the whole interface line to be moved to an optimal position. Cold spots at the very end of the interface line are avoided by an additional low voltage heater (regulated to 400°C) screwed up into the ion source. The reagent gas inlet was arranged in such a way that the CI reagent gas flowed coaxially to the carrier gas into the ion source.

III.2. The Coupling of Glass Capillary Supercritical Fluid Chromatography to a High Temperature GC/MS System

The direct combination of open tubular column supercritical fluid chromatography and mass spectrometry has been reported in a number of papers by Smith et al. (Smith, R.D. et al, 1982a,b; 1983, 1984a,b; 1987) and others (Games, D. et al., 1988, and references cited therein) using both electron impact (EI, Smith, R.D. et al. 1984b) and chemical ionisation (CI, Smith, R.D. et al., 1982,a,b; 1983, 1984a, 1987). In these reports, narrow-bore, fused-silica capillary columns were preferentially used. Direct combination of glass capillary columns, coated with an apolar OH-terminated polysiloxane phase, to a high temperature-GC/MS system was first reported by Blum et al.(Blum, W. et al., 1988b).

III.2.1. The Influence of the Mobile Phase on the Ionisation Process

Each chromatographic method , when combined with a mass spectrometer, can influence the resulting mass spectra to a greater or lesser extent, owing to the influence of the respective mobile phase on the ionisation process. This is of particular importance

when supercritical fluid chromatography is used, and although at first sight the mass spectra from SFC/MS appear similar to spectra from GC/MS, there are certain differences:

i) In contrast to capillary GC, where (with the exception of hydrogen as carrier gas) all mobile phases are inert gases, in SFC, especially in combination with MS, a variety of mobile phases are described which clearly act as reagent gases in positive or negative chemical ionisation [e.g. ammonia, low molecular weight aliphatic hydrocarbons(Wright, H.T. et al.,1985) or methanol which is sometimes used as a modifier (Smith, R.D., 1987)]. This may limit the use of specific reagent gases for structure elucidation, which is of particular interest in geoporphyrin analysis.

ii) In addition, the Joule-Thompson effect makes the control of the ion source temperature in certain circumstances difficult. Cooling increases during a density program which, in SFC, replaces the temperature program normally used in GC analysis. Therefore, the temperature of the ionising plasma varies with the density at which a single component elutes. If the Joule-Thompson effect is too strong (which is likely to occur if packed columns or open tubular columns with i.d. > 0.1 mm are used), the ion source temperature cannot be controlled simply by heating the ion source walls, since the residence time of the sample molecules in the ionising chamber is too short to establish a significant heat transfer.
Owing to the strong temperature dependence of chemical ionisation, the CI-spectra in SFC are only superficially comparable to those obtained from other sample introduction techniques such as GC or direct probe introduction.

iii) The chemical ionisation reaction (e.g. with Bronsted acids like CH_5^+) can even be influenced by inert mobile phases such as the commonly used, carbon dioxide. Although in papers dealing with SFC/MS the influence of carbon dioxide on the CI process has widely been ignored, it can act as a charge exchange (CE) gas.

CE ionisation becomes possible under CI pressure conditions with gases whose ionisation potentials are low but higher than those of the respective organic sample molecules (Hunt, D.F. et al., 1984). If an increasing amount of CO_2 discharges into the CI ionisation chamber during a density program, spectra are obtained which contain additional ions produced (to an increasing extent) as a result of charge exchange. In this respect, the differentially pumped interface system which was recently presented (Smith, R.D. et al.,1987), reduces the influence of the mobile phase, but also the sensitivity.

The interface, which connects the glass capillary SFC column with the MS, is based on a system described by Smith et al. (Smith, R.D. et al., 1982a) but for reasons discussed above, it contains certain modifications. Instead of a capillary restrictor, a polished restrictor (Guthrie, E.G. et al., 1986), which can easily be integrated into the capillary interface, was preferred. In order to optimise and to control the distance between the interface exit and the ionisation chamber, the interface oven (including the interface) was designed to be movable, and adjustable. This allows, if necessary, the automatic focusing of the interface exit during a given density program in order to keep the influence of the Joule-Thompson effect on the ion source temperature and the contribution of the mobile phase as constant as possible. In SFC/MS, the amount of mobile phase is reduced by using capillary columns of i.d. < 0.1 mm. The loss of dynamic range of the narrower bore columns was compensated by thicker coatings.

Concerning the interface, we adopted the coaxial dual gas principle, which has been successfully used in GC/MS for many years, and introduced the CI reagent gas parallel to the chromatographic effluent into the ion source as shown in Fig.17 below.

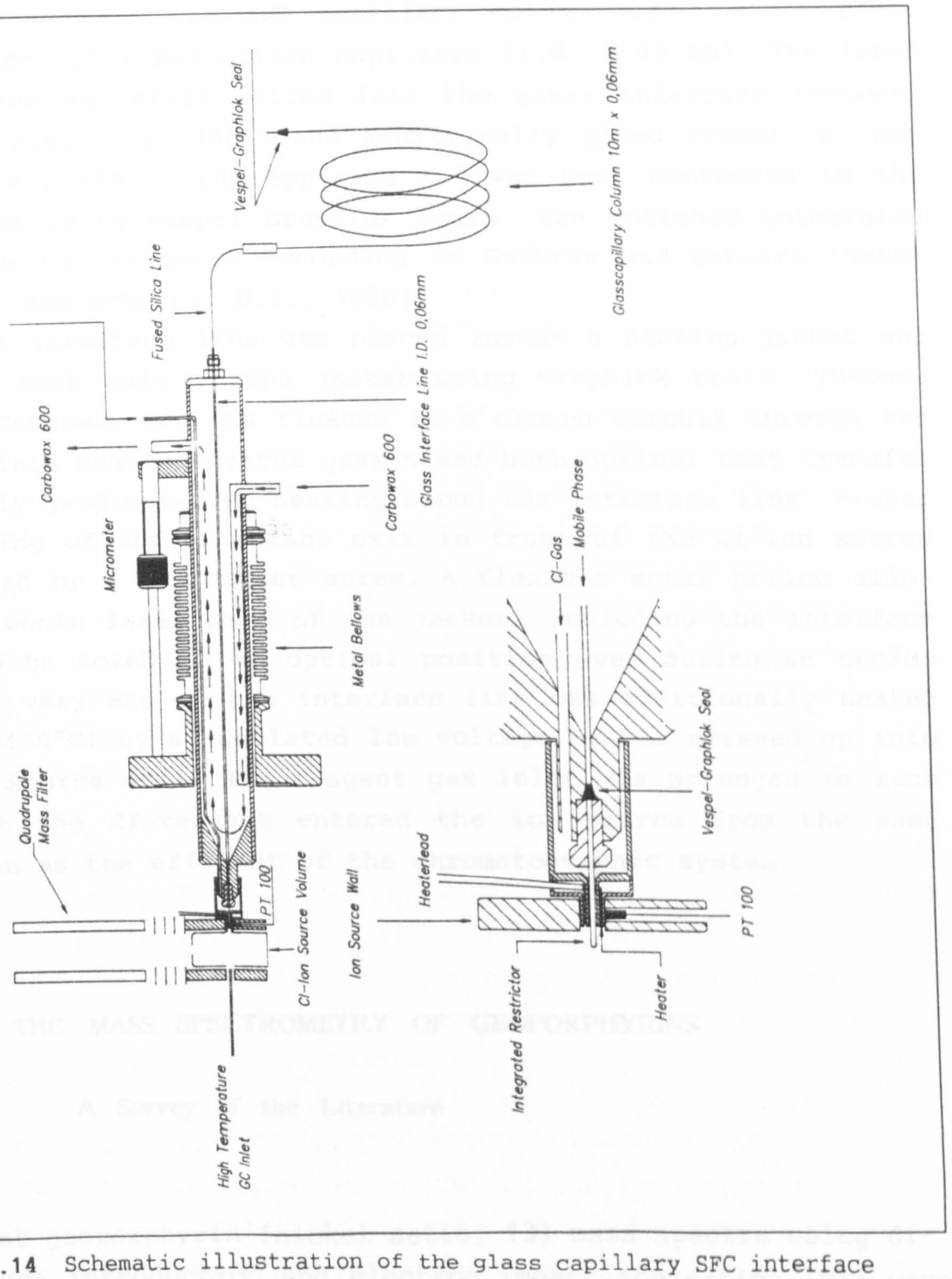


Fig.14 Schematic illustration of the glass capillary SFC interface linked to the GC/MS system. The enlarged representation below shows the entrance into the ionisation chamber, with additional electrical heater and the CI-reagent gas inlet parallel to the interface capillary. From Blum et al.,**1988b**

The glass interface line with an i.d. of 0.06 mm and an o.d. of 0.8 mm extends to the SFC capillary column via a short piece (about 5 cm) of fused silica capillary (i.d. 0.05 mm). The fused silica line was press fitted into the glass interface (Rohwer, E.R. and Pretorius, 1987) and additionally glued (Vecci, M. and Walther, W., 1987). The opposite end was butt connected to the SFC column using Vespel Graphlok seals. The polished integrated restrictor was prepared according to Guthrie and Schwarz (Guthrie, E.G. and Schwarz, H.E., 1986).

The glass interface line was placed inside a heating jacket and fixed on both ends of the jacket using Graphlok seals. Thermostatted Carbowax 600 was flushed in a closed circuit through the jacket. This heating system guaranteed both optimal heat transfer and nearly gradient-free heating along the interface line. Proper positioning of the interface exit in front of the CI-ion source is ensured by a micrometer screw. A flexible metal bellow allowed the whole inner part of the jacket, including the interface line, to be moved to an optimal position even during an SFC/MS run. The very end of the interface line was additionally heated (to 150-400°C) by a regulated low voltage heater screwed up into the ion source wall. The reagent gas inlet was arranged in such way that the CI-reagent entered the ion source from the same direction as the effluent of the chromatographic system.

IV. THE MASS SPECTROMETRY OF GEOPORPHYRINS

IV.1. A Survey of the Literature

The first geoporphyrin (nickel aetio, 13) mass spectra using direct probe introduction and electron impact ionisation (EI) were reported in 1960 by Hood et al. (Hood, A. et al., 1960) and one year later (vanadyl aetio, 14) by Mead et al. (Mead, W.L. and Wilde, A.J., 1961). Dean et al. (Dean, R.A. and Whitehead, E.V., 1963) presented the first mass spectrometrical evidence for the presence of a number of vanadyl alkylporphyrins in the range of C_{28}-C_{33} in different oil shales.

Thomas and Blumer (Thomas, D.W. and Blumer, M., 1964) investigated extracts from Serpiano oil shales by EI-MS after having isolated several vanadyl porphyrins by thin layer chromatography (TLC). In that work a large number of vanadyl alkylporphyrins, and the corresponding chlorins could be identified. Baker (Baker, E.W., 1966) published the identification of geoporphyrins from asphaltenes (for the first time the rhodo(benzo)porphyrin (15) type was identified). The free bases described in this study were obtained after extraction of the metalloporphyrin with methanesulphonic acid of the asphaltenes.

In order to confirm the empirical formula of aetio- and deoxophyloerythroetioporphyrin (DPEP)(16) accurate mass measurements were carried out by Baker et al.(Baker, E.W. et al., 1967) and this method was applied in the investigation of a number of asphaltenes from sands and oil shales. From these investigations a diagenetic ring closure followed by an aromatisation was suggested as a possible mechanism for the formation of alkylbenzoporphyrins. By means of fractionated evaporation in the 300-500°C range and high resolution mass spectrometry evaporation profiles of low molecular weight and high molecular weight porphyrins could be obtained from Boscan asphaltene fractions (Gallegos, E.J., 1967). Vanadyl porphyrin complexes with molecular weights above 900 daltons from Serpiano oil shales were reported by Blumer et al. (Blumer, M. and Snyder, W.D., 1967; Blumer, M. and Rudrum, M.J., 1970). From the volatilisation behaviour of the high molecular weight fractions they derived the possibility of the presence of dimeric porphyrins in oil shales.

Alturki et al. (Alturki, Y., Eglinton, G. and Pillinger C.T., 1975) confirmed that Gilsonite bitumen shows a series of two major structural types, aetio and DPEP, and that each series was separable into a number of components of different polarity, whereby each structural type made a group of compounds that shares the tetrapyrrolic macrocycle but differs in the nature of their alkyl substitution pattern. These investigations were continued by Didyk et al. (Didyk, B., Alturki, Y.I., Pillinger, C.T. and Eglinton, G., 1975). In a series of reports, appearing between 1970-1975 Baker (e.g. Baker, E.W. et al.,1970) investi-

gated petroporphyrins found in core samples obtained by the Deep Sea Drilling Project (DSDP). The aim of these studies was to get a continuous history of the chemical degradation of chlorophylls to the geoporphyrins.

The unexpected co-occurance of free base chlorins and vanadyl porphyrins found in DSDP-samples was explained by a mixing of older and younger sediments (Smith, C.D. et al.,1974). The mass spectrometrical evidence for both free base DPEP- (16) and aetioporphyrins (1) in addition to chlorins (17) or isochlorins (18) was reported by Casagrande et al. (Casagrande, D.J. et al.,1976). Palmer et al. reported similar observations (Palmer, S.E. et al., 1979). Direct probe investigations of crudes from different sources like Bachaguero, Venezuela (Nuzzi, et al., 1974), Romashkino, Czechoslovakia (Sebor, G. et al., 1980) and from different places in Russia (Serebrennikova, O.V. et al, 1975; Antipenko, V.P. et al., 1978; Burkova, V.N. et al., 1978) were in accordance with earlier investigations but they also showed the limitation of direct probe mass spectrometry in handling the complex mixtures of naturally occuring geoporphyrins.

Eglinton and colleagues (Eglinton, G. et al., 1979a; Quirke, J.M. E. et al.,1979) tried to overcome this problem. They followed a way pointed out by Thomas and Blumer (Thomas, D.W. and Blumer, M., 1969) by introducing a chromatographic separation step prior to MS analysis. By means of high pressure liquid chromatography (HPLC) they isolated several aetio-(1) and DPEP (16) porphyrins in single carbon number fractions and using both, MS and NMR as spectroscopic methods, they were able to elucidate the full structure of four geoporphyrins. Since then this approach has become the most successful way in order to elucidate the structures of unknown geoporphyrins.

IV.2. Structure Elucidation of Geoporphyrins by means of Mass Spectrometry

IV.2.1. Free Base Porphyrins.

IV.2.1.1. Electron Impact Ionisation Mass Spectrometry of Free Base Porphyrins

The EI mass spectra of free-base geoporphyrins exhibit little cleavage of the macrocyclic ring system and the molecular ion is dominant. The fragmentation is more or less limited to peripheral substituents and, in addition, the spectra show doubly-charged molecular ions and doubly-charged fragment ions in the lower mass range. A typical EI mass spectrum of a free base standard porphyrin is shown in Fig.15 below. EI spectra give the important molecular weight information from which the empirical formula can be derived.

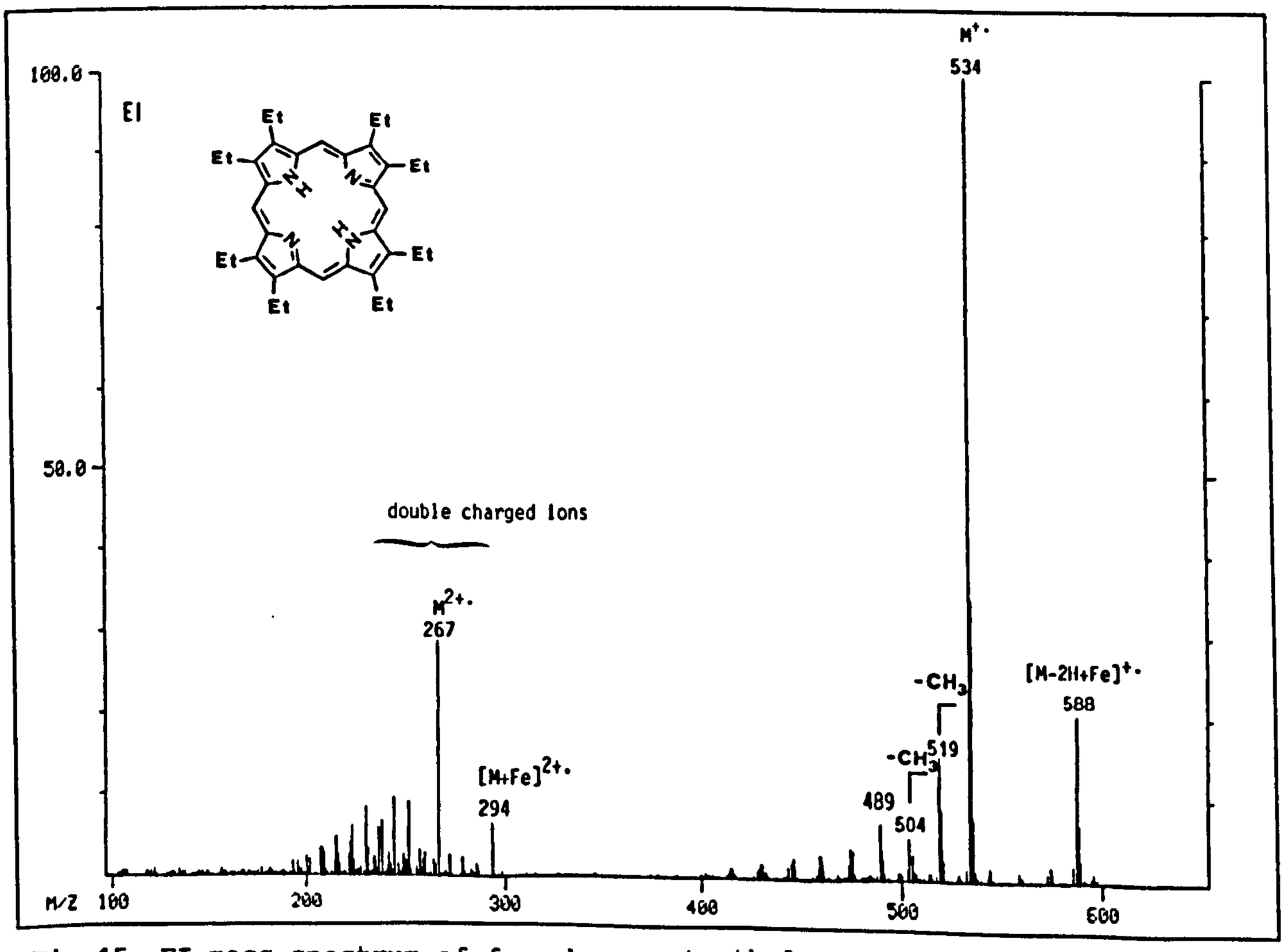


Fig.15 EI-mass spectrum of free-base octaethylporphyrin. Introduction system: capillary column. For experimental conditions see Section VII.2.1.-2.2..

While the presence of alkyl-substituents can be derived from the molecular ion, the location of the substituent on a particular pyrrole ring and its sequence in the tetrapyrrole system is in general not accessible.

Moreover, the structure analysis of geoporphyrins has for practical reasons to be carried out in mixtures containing a variety of different porphyrin homologues and isomers. But even if a chromatographic system is directly coupled to the mass spectrometer in order to separate these mixtures, a chemical pretreatment involving either oxidative (Williamson, A.L. and Ellworth, R.K., 1971; Jackson, A.H. et al., 1974; 1980) or reductive(Maines, M.D. and Anders, M.W., 1973; Stoll, M.S. and Elder, G.H., 1973) degradation of the porphyrins and subsequent analysis of the degradations products by means of GC/MS cannot be used, since the assignment of the degradation products to a particular single mixture component is not possible.

Another avenue involving the conversion of free-base geoporphyrins to derivatives is the reduction with Raney-Ni as a catalyst to porphyrinogens which fragment more readily. This was shown by Budzikiewicz et al. (Budzikiewicz, H. and Pesch, R.,1976). Porphyrinogens (4) fragment under EI conditions into ions containing one, two and three pyrrole rings (see Fig.16). As already discussed in Section I.1.1. (Fig.2) porphyrinogens could be excellent derivatives for GC/MS investigations, owing to their low retention temperatures. Unfortunately, they are very sensitive to oxidation and unstable in solution even when protected under argon.

IV.2.1.2. Chemical Ionisation Mass Spectrometry of Free Base Porphyrins

Eglinton, et al. (Eglinton, G. et al.1979b; Shaw, G.J. et al. 1978; Shaw, G.J. et al. 1981) were the first to demonstrate that porphyrinogens are also produced in the ion source of a mass spectrometer during chemical ionisation. They used CI reagent gases with different proton affinities (e.g. CH_4 or H_2), and showed that fragmentions with one, two and three pyrrole rings can also be observed directly.

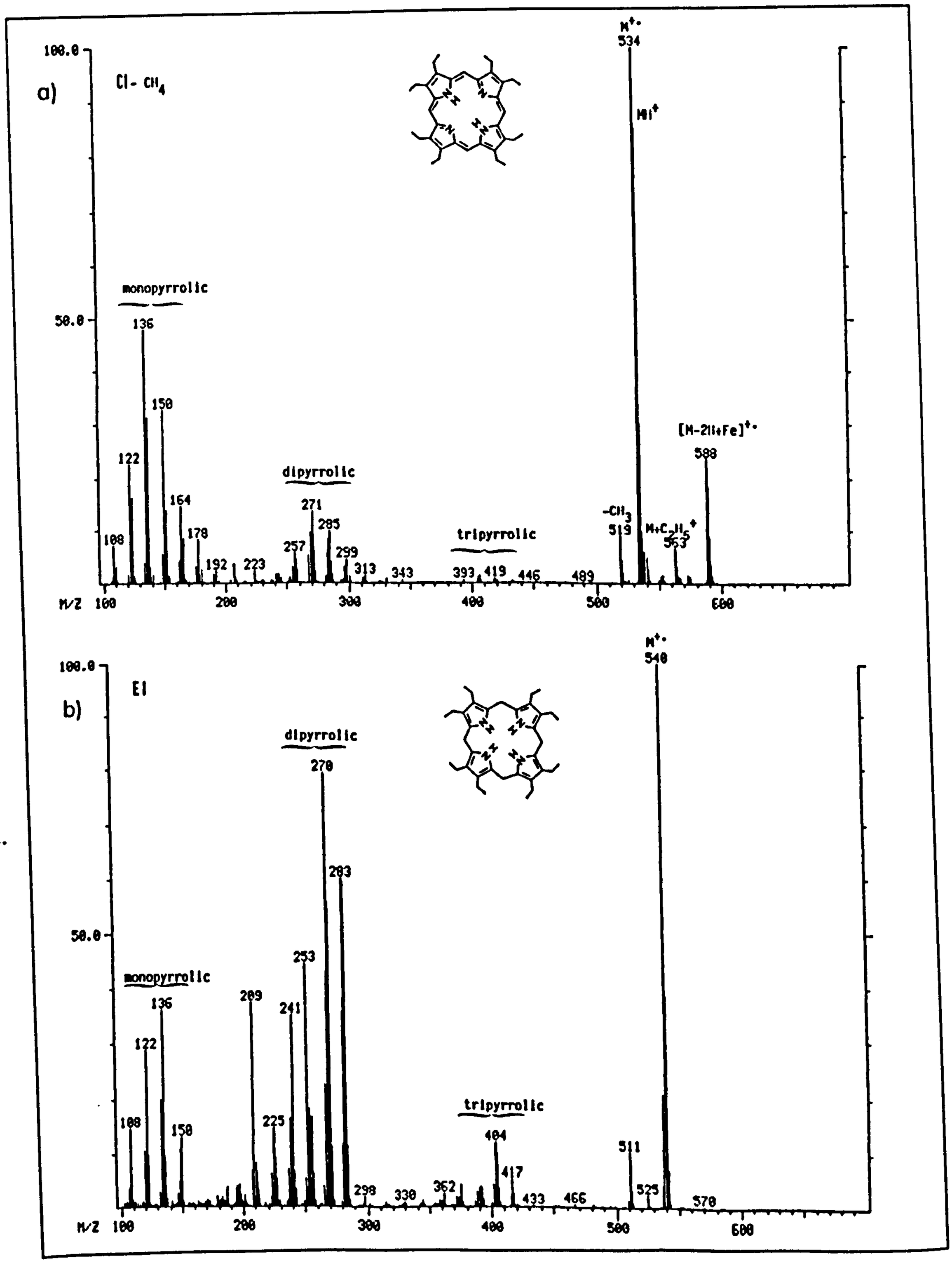


Fig.16 Similarity of the octaethylporphyrin mass spectrum under CI/CH_4 conditions (trace a) and of the corresponding porphyrinogen under EI condition (trace b). Introduction: capillary column. For experimental details see Section VII.2.1..

In parallel with the EI mass spectrum of octaethylporphyrinogen (Fig.16, trace b) extensive fragmentation occurs resulting in three triads of peaks in each of the mono-, di- and tripyrrole regions. In addition, the molecular ion region of the CI/CH_4 MS (Fig.16, trace a) exhibits a cluster of peaks containing the MH^+-ion and $(M + 2H + H)^+$, $(M + 4H + H)^+$ and $(M + 6H + H)^+$ representing the different degrees of hydrogenation of the porphyrin molecular ion. The $(M + 6H + H)^+$-ion has four additional hydrogens in the meso positions and two additional imido hydrogens. The principal fragmentation pathway for octaethylporphyrin in CI-mode is derived from Shaw et al. (Shaw, G.J.; Eglinton, G. and Quirke, J.M.E., 1981) and shown in the Figure below.

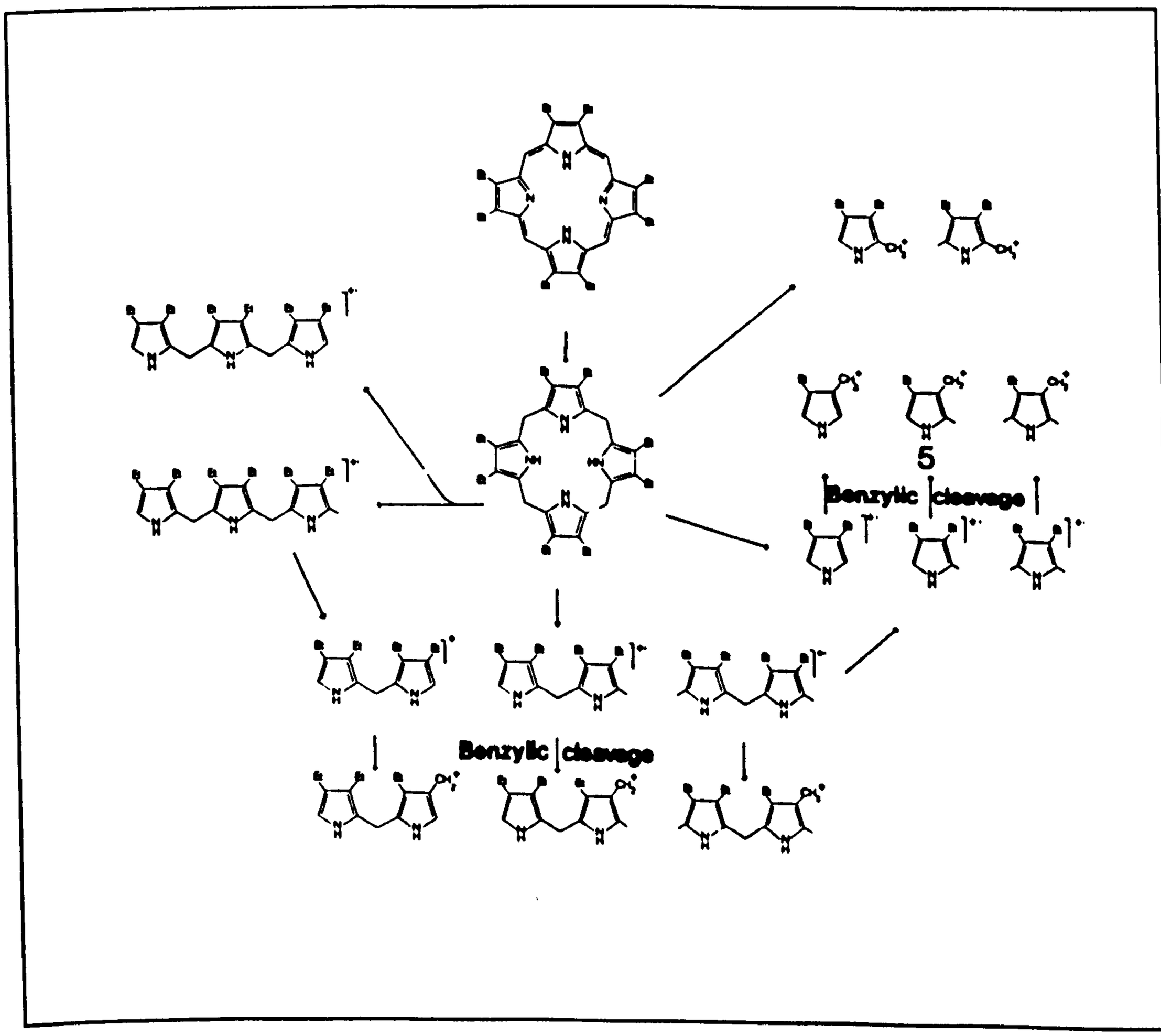
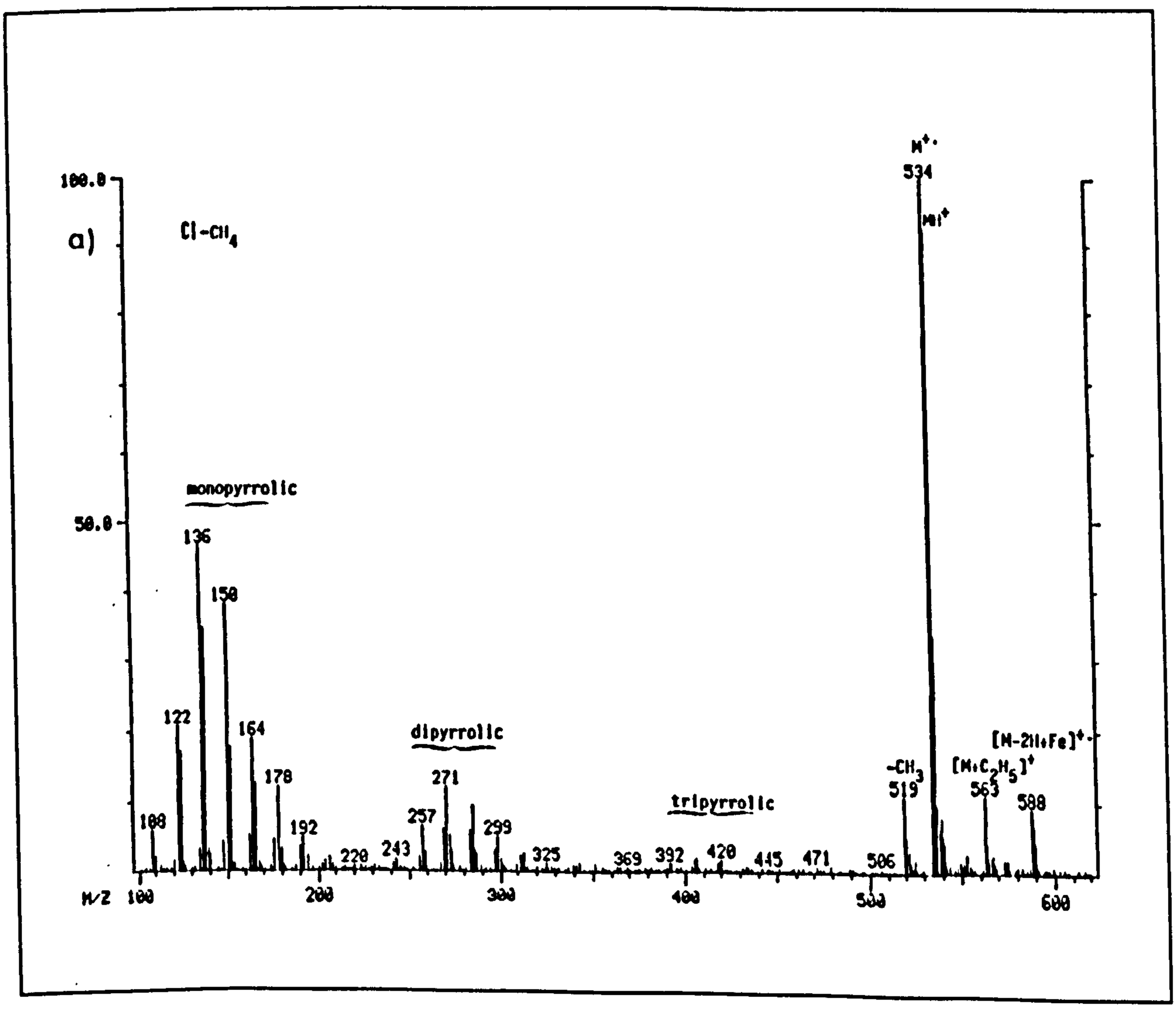


Fig.17 Principal pathway to key mono-, di- and tripyrrolic ions of octaethylporphyrin under CI/H_2 conditions. From Shaw et al., 1981.

IV.2.1.2.1. Selection of the Chemical Ionisation Reagent Gas

Three reagent gases, methane (Shaw,G.J. et al., 1978), hydrogen (Shaw, G.J. 1981; Shaw, G.J. et al., 1981; Wolff,G.A., 1983; 1984; Sundaraman, P. et al., 1984; Evershed, R.P.,1985) and ammonia (Jiang,X.Y. et al,1984; Tolf, B.R. et al.,1986) have been employed in chemical ionisation mass spectrometry for structure elucidation of free base alkylporphyrins so far. Although the reported fragmentation pathways are similar, the relative abundance of the structurally relevant fragment ions are different in each case. This is shown in Fig.18.



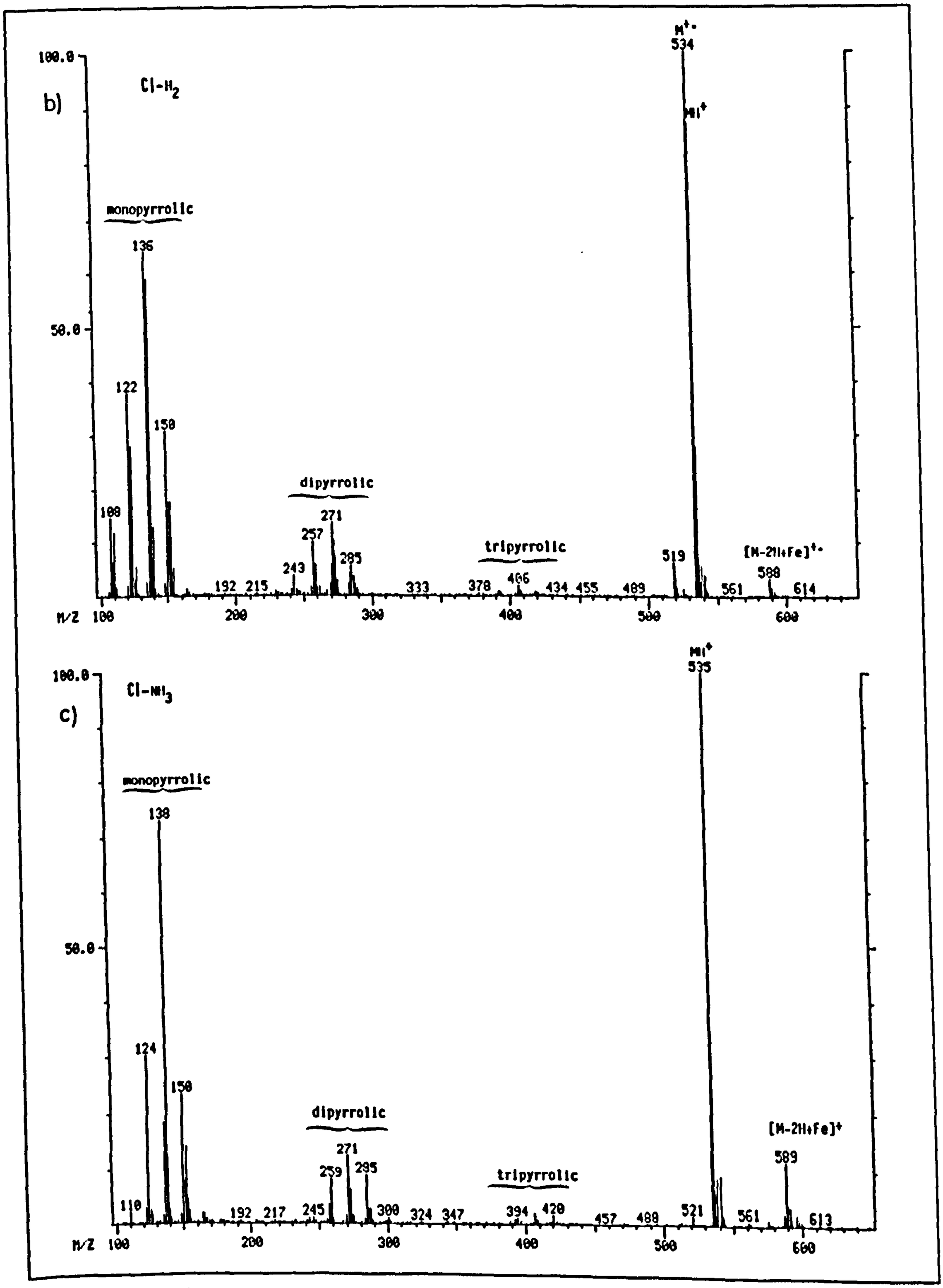


Fig.18 a) CI/CH_4 mass spectrum of octaethylporphyrin, b) CI/H_2 mass spectrum of octaethylporphyrin, c) CI/NH_3 mass spectrum of octaethylporphyrin. Introduction: capillary column. For experimental conditions see Section VII.2.1..

Two main fragmentation pathways of $(M + 6H + H)^+$-ions generally obtained in chemical ionisation spectra of porphyrins are illustrated in the two next figures. Octaethylporphyrin was used as an example since it contains four equivalent substituted pyrrole rings and exhibits quite clearly the fragmentation behaviour of porphyrins under CI conditions.

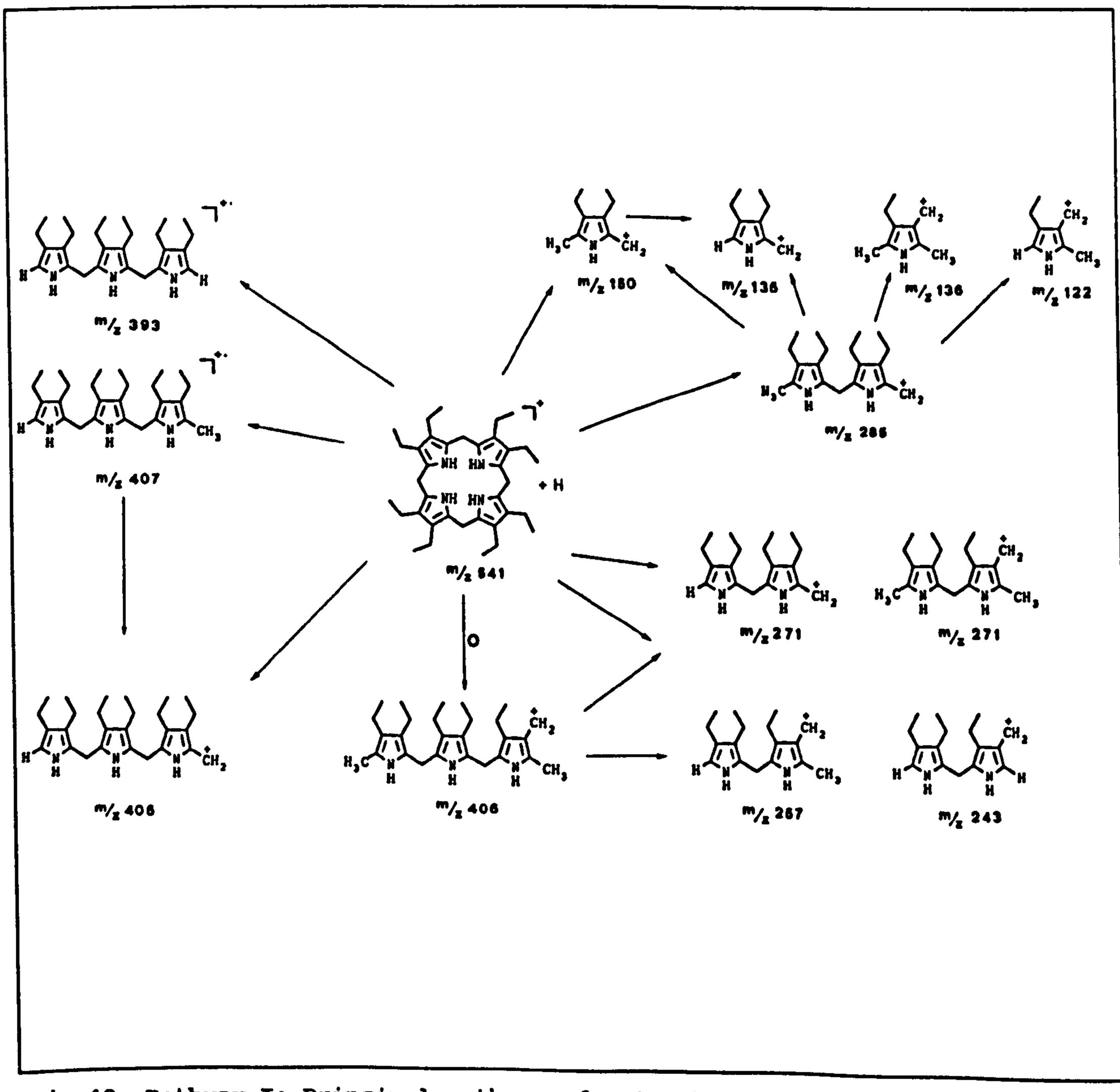


Fig.19 Pathway I: Principal pathway of octaethylporphyrin under CI/H_2 conditions. Modified from Shaw et al. 1981.

Pathway I: The $(M + 6H + H)^+$-ion (porphyrinogen) undergoes a fragmentation in one of the meso positions by formation of odd-electron monopyrrolic, dipyrrolic and tripyrrolic ions. This fragmentation is similar to that postulated for porphyrinogens under EI-conditions (Budzikiewicz and Pesch, 1976). The latter ions undergo further fragmentation by α-cleavage and form more stable secondary fragments (even electron). These secondary ions are also produced directly from $(M + 6H + H)^+$-ions or by a benzylic cleavage of tri- or dipyrrolic ions and leads to the most dominant fragments at m/z 122, m/z 136 and m/z 150 in the mono-pyrrolic range and at m/z 271 and m/z 285 in the dipyrrolic range.

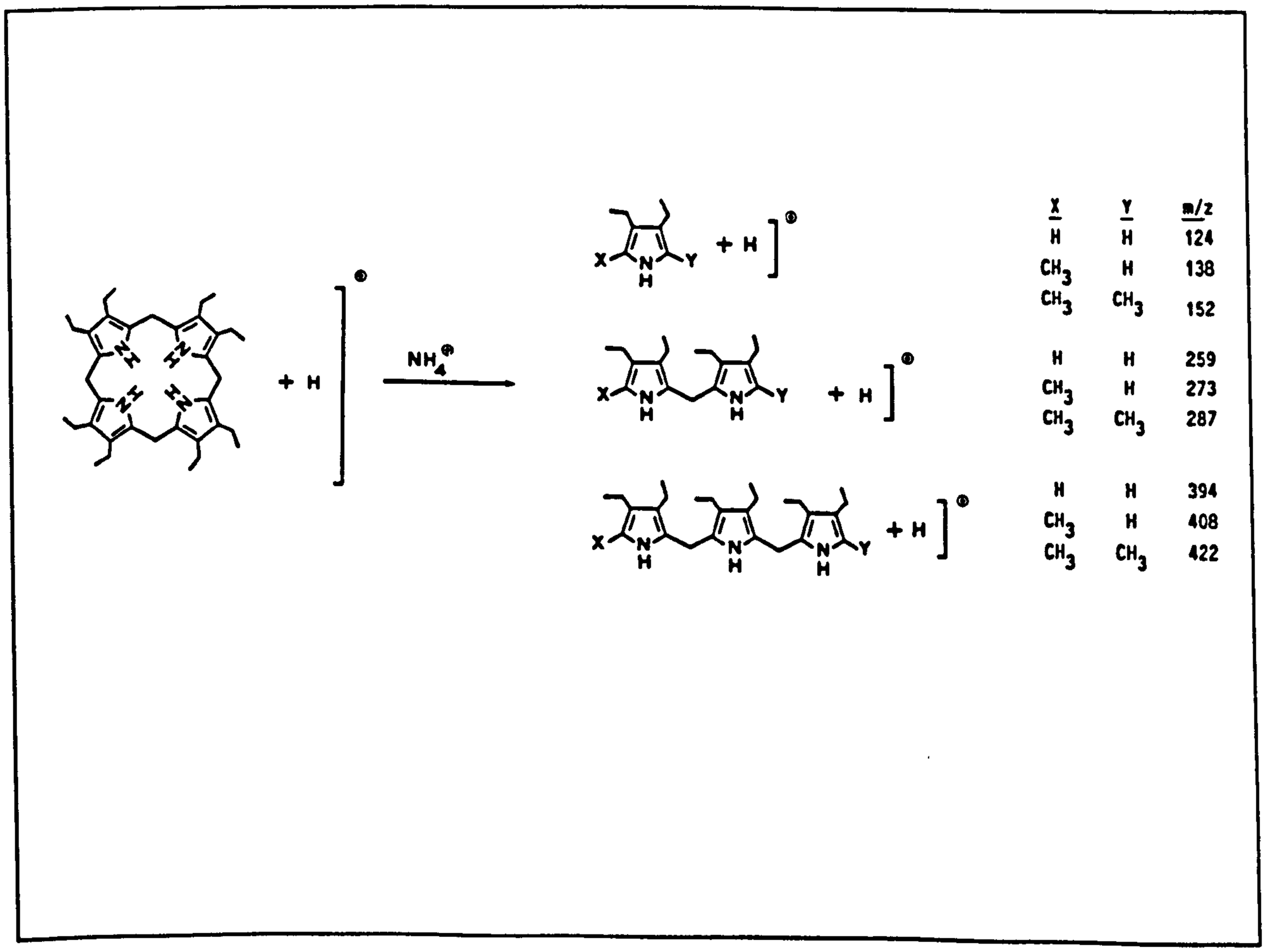


Fig.20 Pathway II: Principal fragmentation pathway for octaethylporphyrin under CI/NH_3 conditions. Modified from Tolf et al. 1986.

Pathway II is more easily appreciated and is of importance when reagent gases with high proton affinities are used. In NH_3-CI the $(M + 6H + H)^+$- ions also undergo fragmentation in meso positions followed by the formation of mono-, di- and tripyrrolic fragments. In contrast to pathway I, most of the ions are protonated (even-electron), particularly in the monopyrrole range and the secondary ion formation is reduced (see Fig.18c). The contribution of the ions of these two pathways appears to depend on the proton affinity of the respective reagent gases. In the case of hydrogen (proton affinity 101 kcal mol^{-1}) as CI-reagent gas pathway I is dominant. With ammonia (proton affinity 207 kcal mol^{-1}) pathway II prevails and pathway I is less important.
In addition to that obtained under EI the following structural information can be obtained from CI-mass spectra, independent of the nature of the reagent gases:

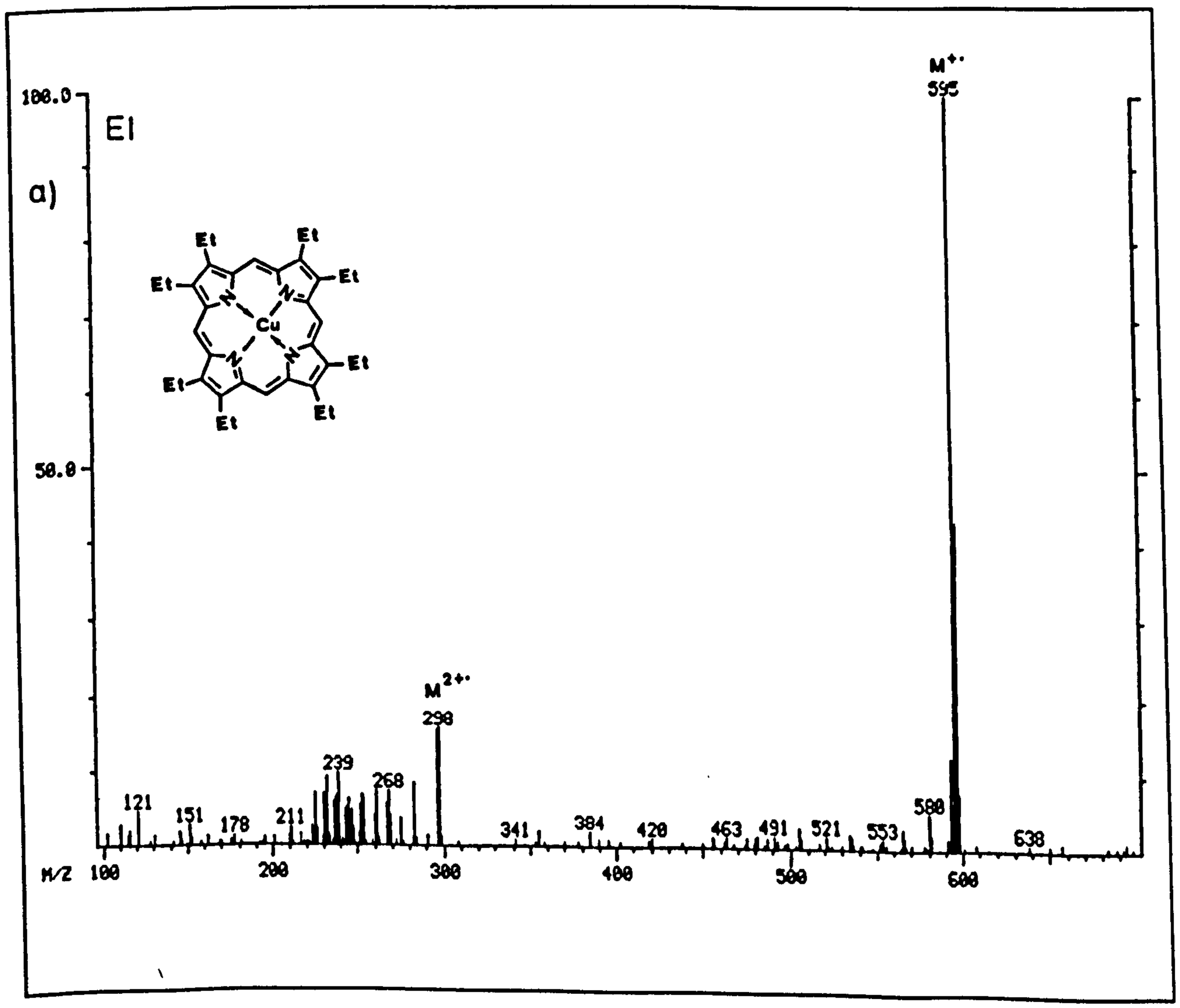
- The extent of alkyl substitution of a single pyrrole.
- The arrangement of the pyrrole rings in the original porphyrin.

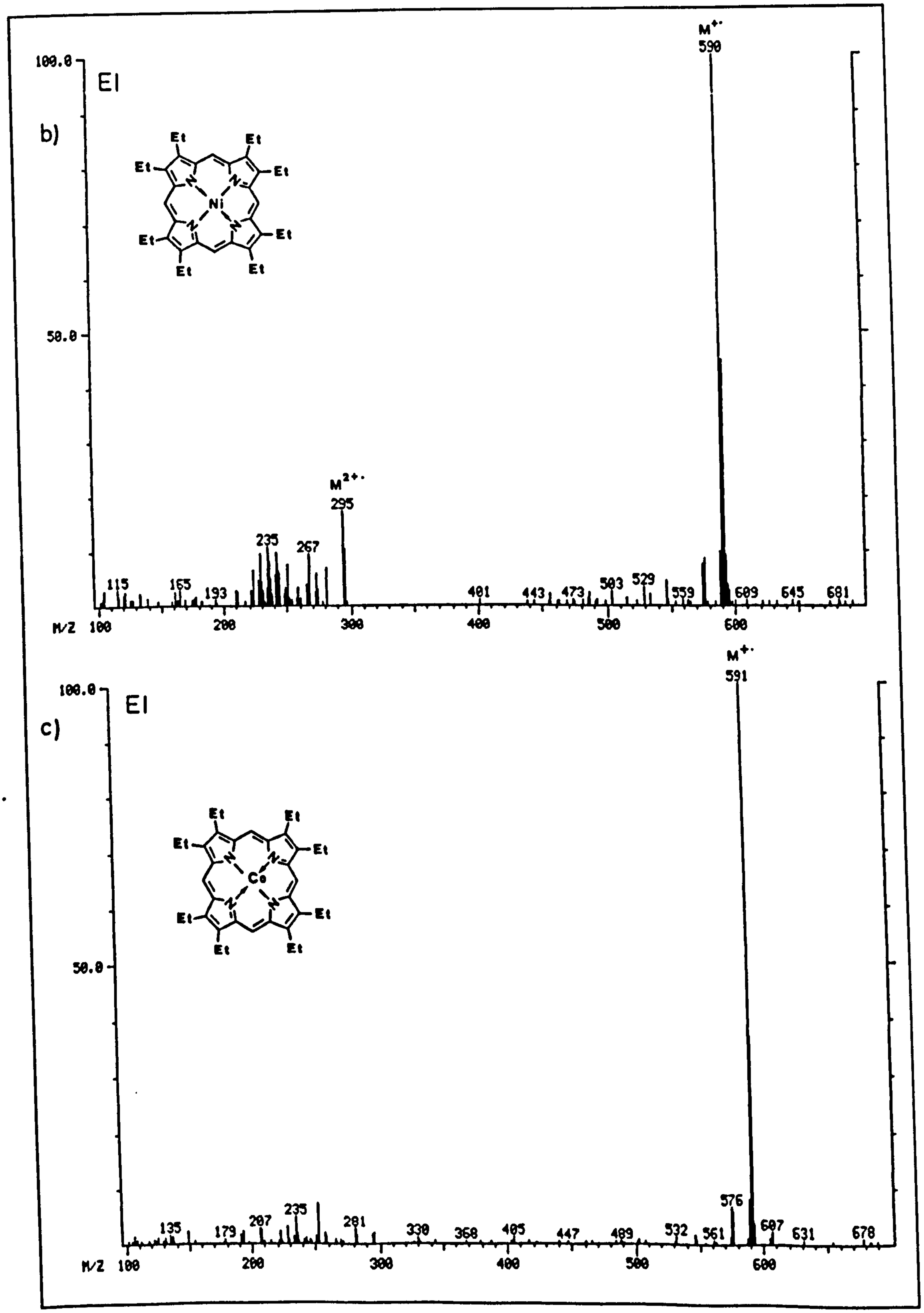
But neither the exact nature of a substituent (e.g. two methyl groups can not be distinguished from one ethyl group) nor the true orientation of a single pyrrole ring in the tetrapyrrole macrocycle can be derived from mass spectral data (Shaw et al. 1981).
Thus, for the characterisation of single GC peaks and discussion of the results in chapter five, the semi-systematic nomenclature of Yen et al. (Yen, T.F. et al., 1969), in which alkylporphyrins are classified by their molecular weight, has been used as modified by Gill (Gill, J.P.,1984d).

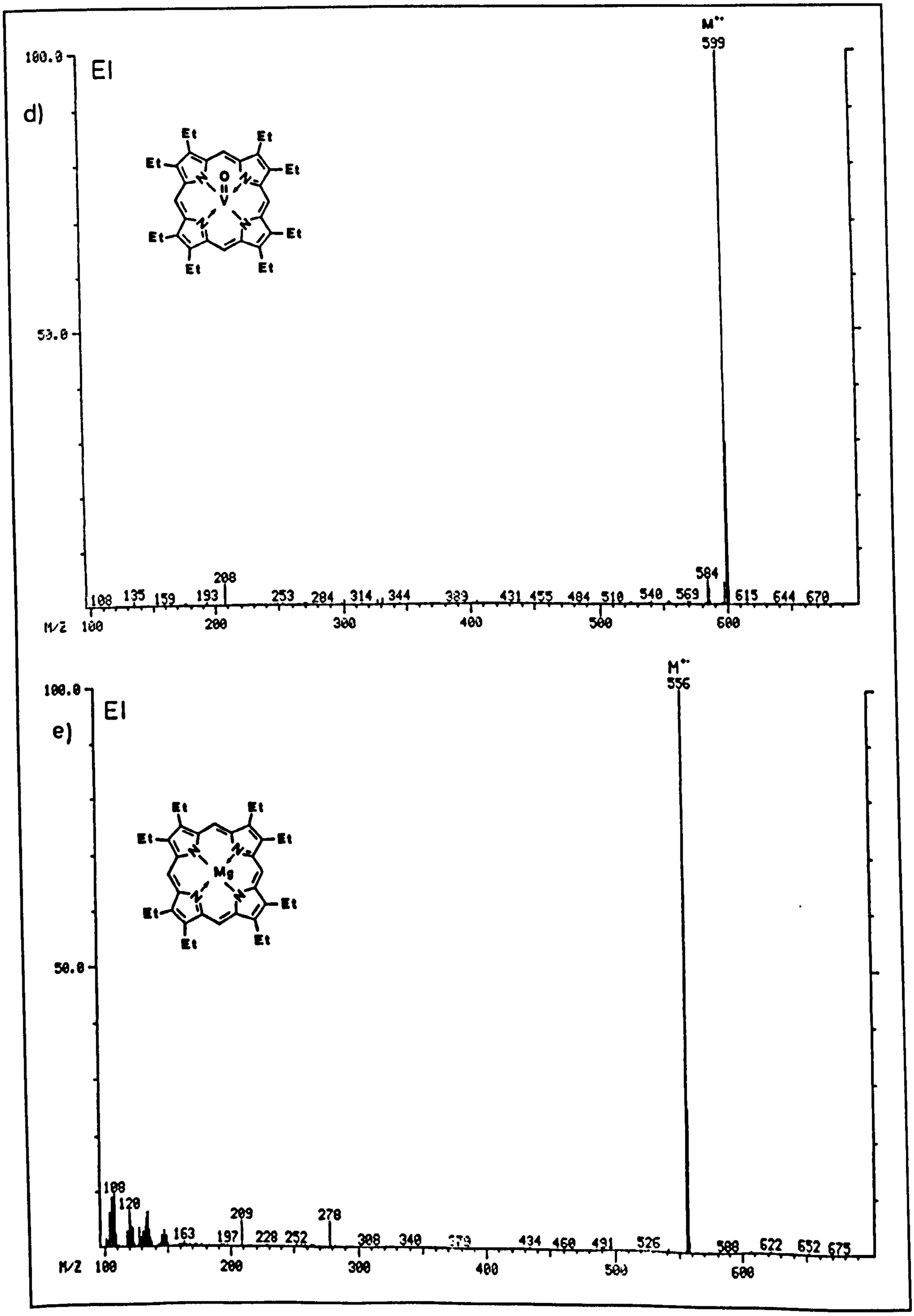
IV.2.2 Metalloporphyrins

IV.2.2.1. Electron Impact Mass Spectrometry of Metalloporphyrins

The EI mass spectra of metalloporphyrins are similar to those obtained from free base porphyrins. Since the metal is not lost during the fragmentation process, the dominant ion is the molecular ion. Fragmentation of peripheral substituents and the formation of doubly charged ions are of minor importance and analytical relevance. The similarity among different metalloporphyrin EI mass spectra is illustrated with the example of copper-, nickel-, cobalt-, vanadyl-, zinc- and magnesium-octaethylporphyrin in Fig.21a - f. (Each metall porphyrin was run as a separate sample. Zn- and Mg-octaethylporphyrin did not appear after chromatography when injected as a mixture with other metalloporphyrin standards).




b)
EI
100.0
50.0
Et
Ni
M2+·
295
M+·
590
115
165
193
235
267
401
443
473
503
529
559
609
645
681
M/Z
100
200
300
400
500
600
c)
EI
100.0
50.0
Co
M+·
591
135
179
207
235
281
330
368
405
447
489
532
561
576
607
631
678
M/Z
100
200
300
400
500
600


d)
EI
100.0
53.0
M••
599
Et
O
V
N
208
584
108 135 159 193 253 284 314 344 389 431 455 484 510 540 569 615 644 670
M/Z 100 200 300 400 500 600
e)
EI
100.0
50.0
M••
556
Mg
108
120
209
278
163 197 228 252 308 340 379 434 460 491 526 588 622 652 675
M/Z 100 200 300 400 500 600

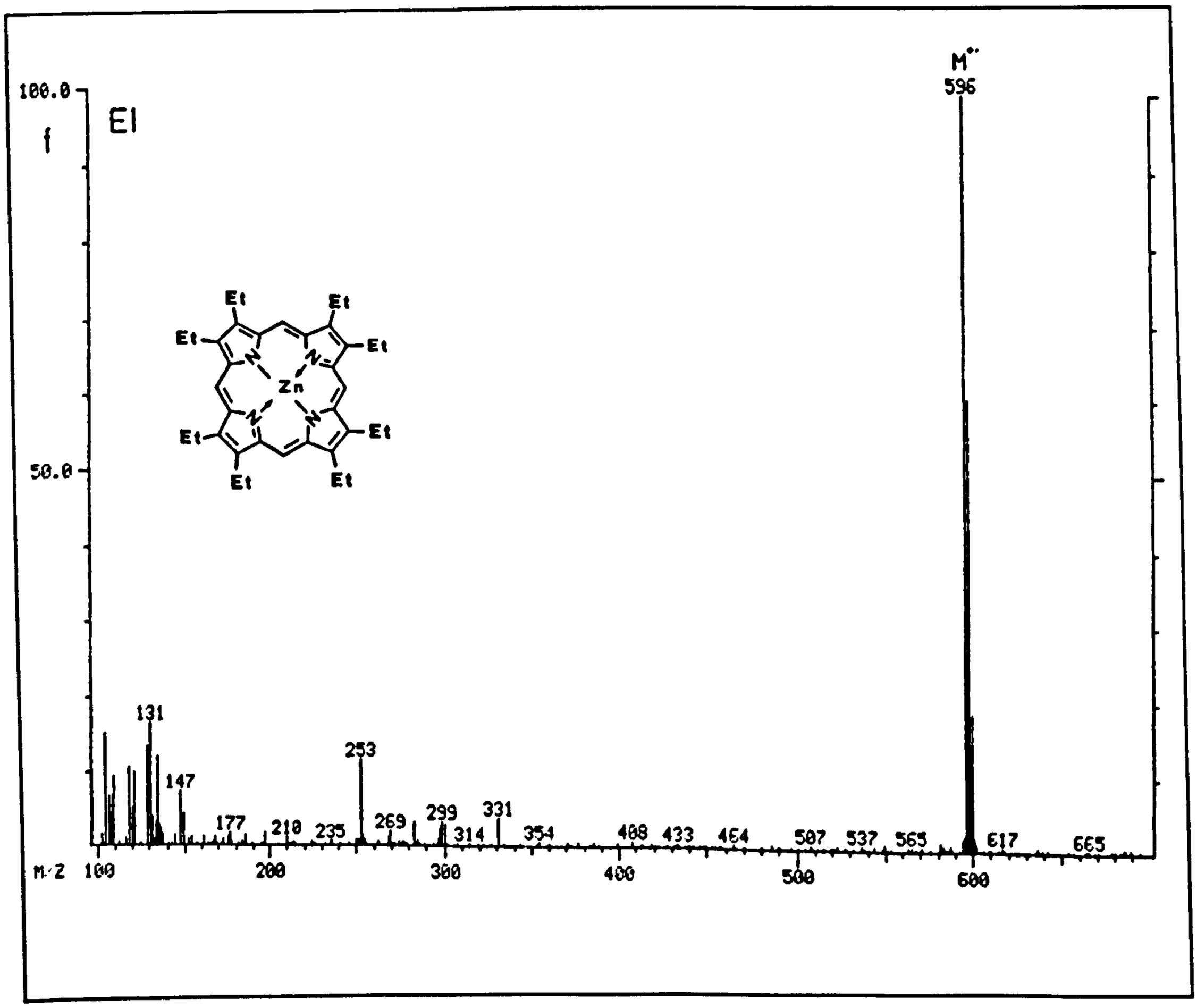


Fig.21 EI mass spectrum of a) Cu(II)octaethylporphyrin, b) Ni(II)octaethylporphyrin, c) Co(II)octaethylporphyrin, d) V(IV)octaethylporphyrin, e) Mg(II)octaethylporphyrin and f) Zn(II)octaethylporphyrin. Introduction system: capillary column. For experimental details see Section VII.1.3, 2.1 and 2.2.. Note that the M^{2+} -ions showed different rel.intensities.

The main information from the metalloporphyrin EI mass spectra is the identification of the chelating metal by the mass difference to the respective free-base or/and by the isotopic pattern of the M^{+}·-ion, respectively. The isotopic clusters of some geochemically important metallo-geoporphyrins are exemplified in Fig.22 where the enlarged molecular ion patterns of chelated octaethylporphyrins are shown.

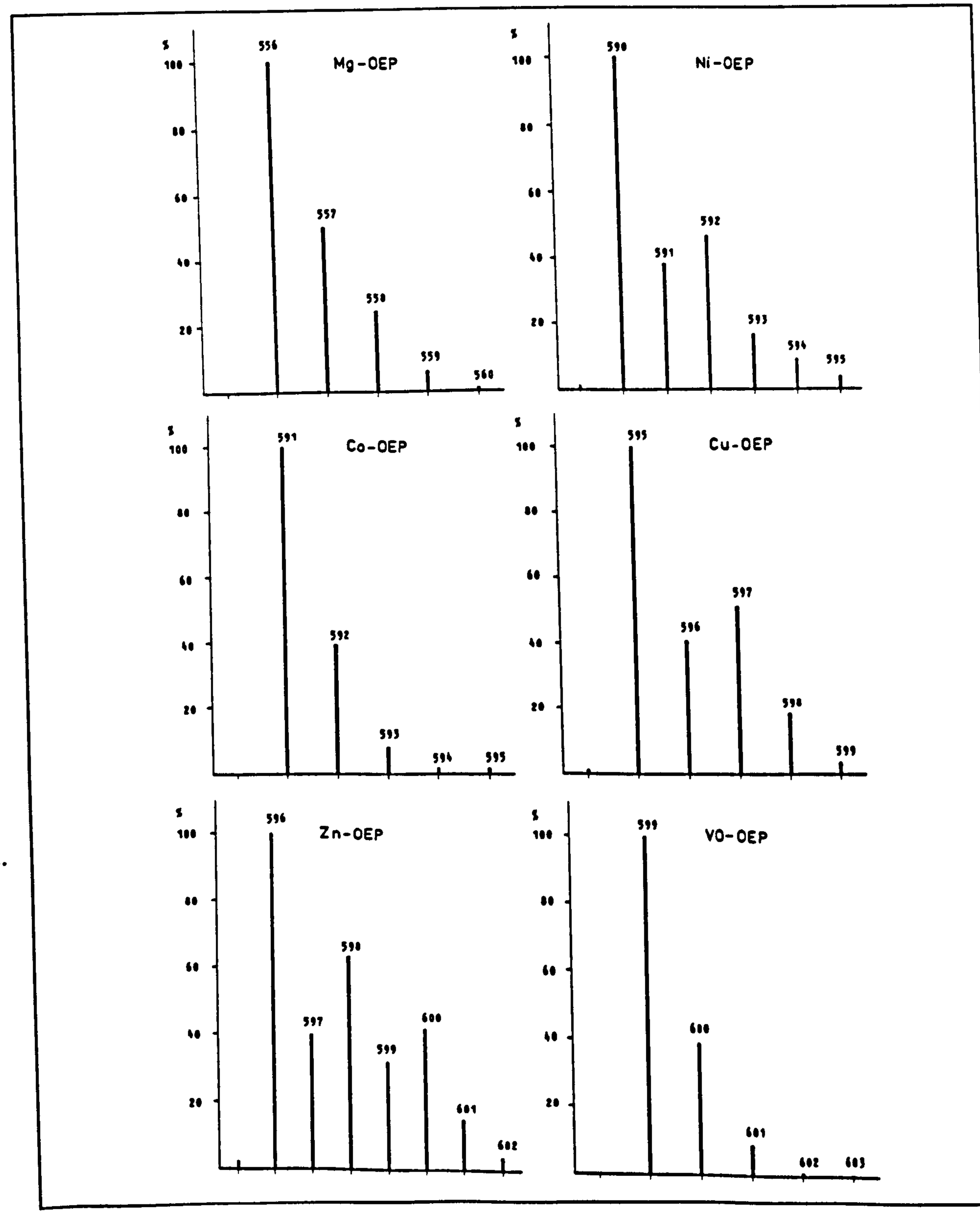
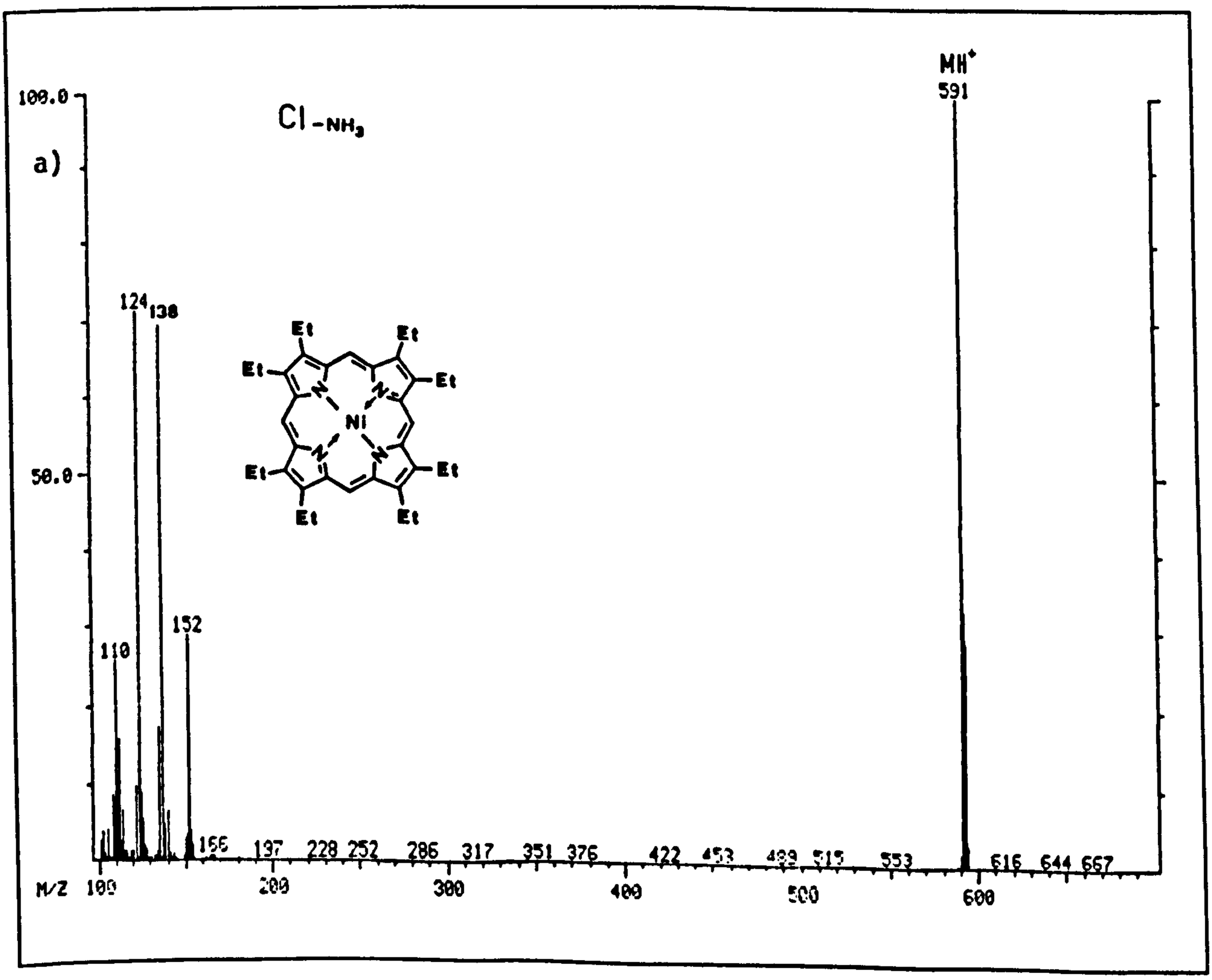
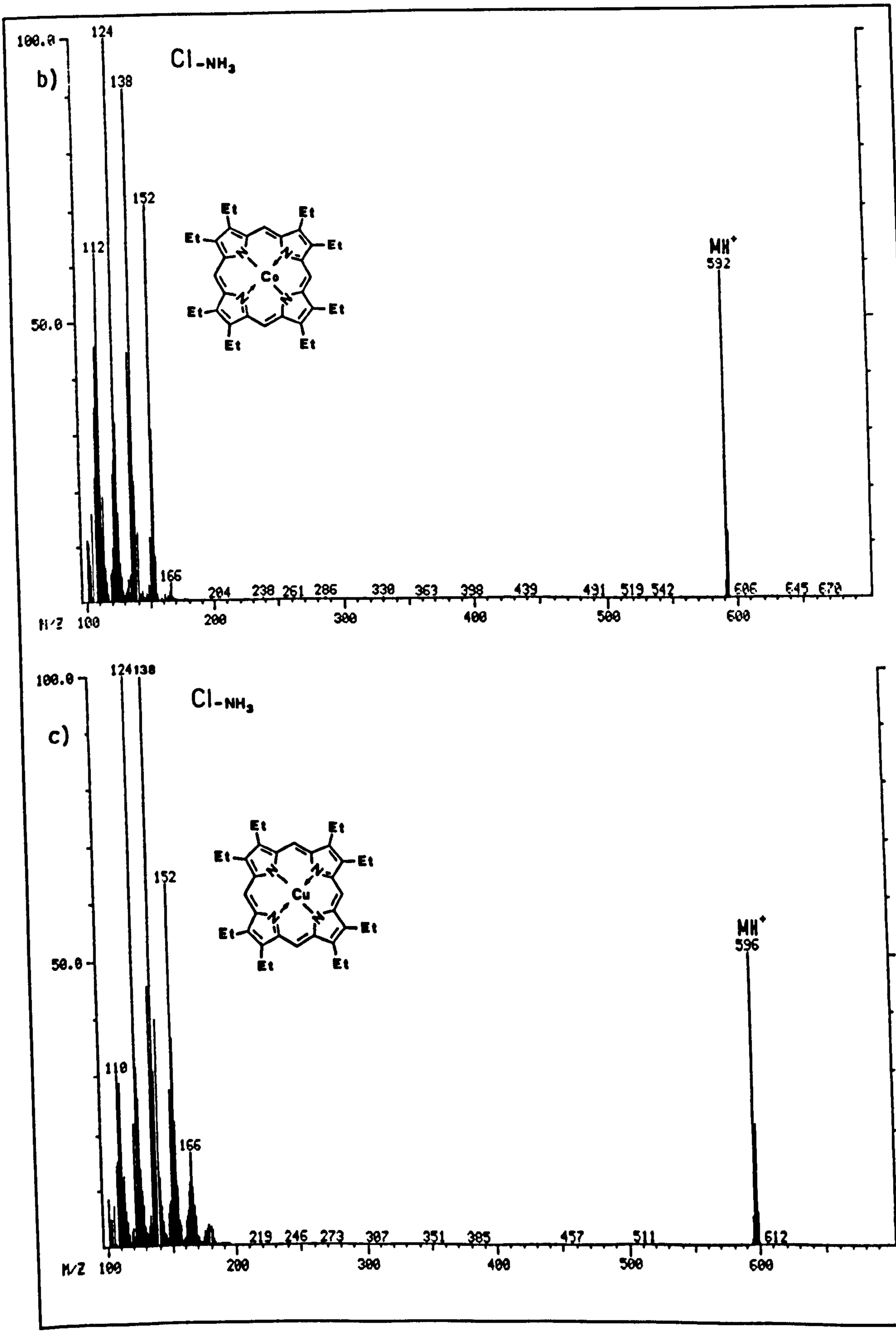


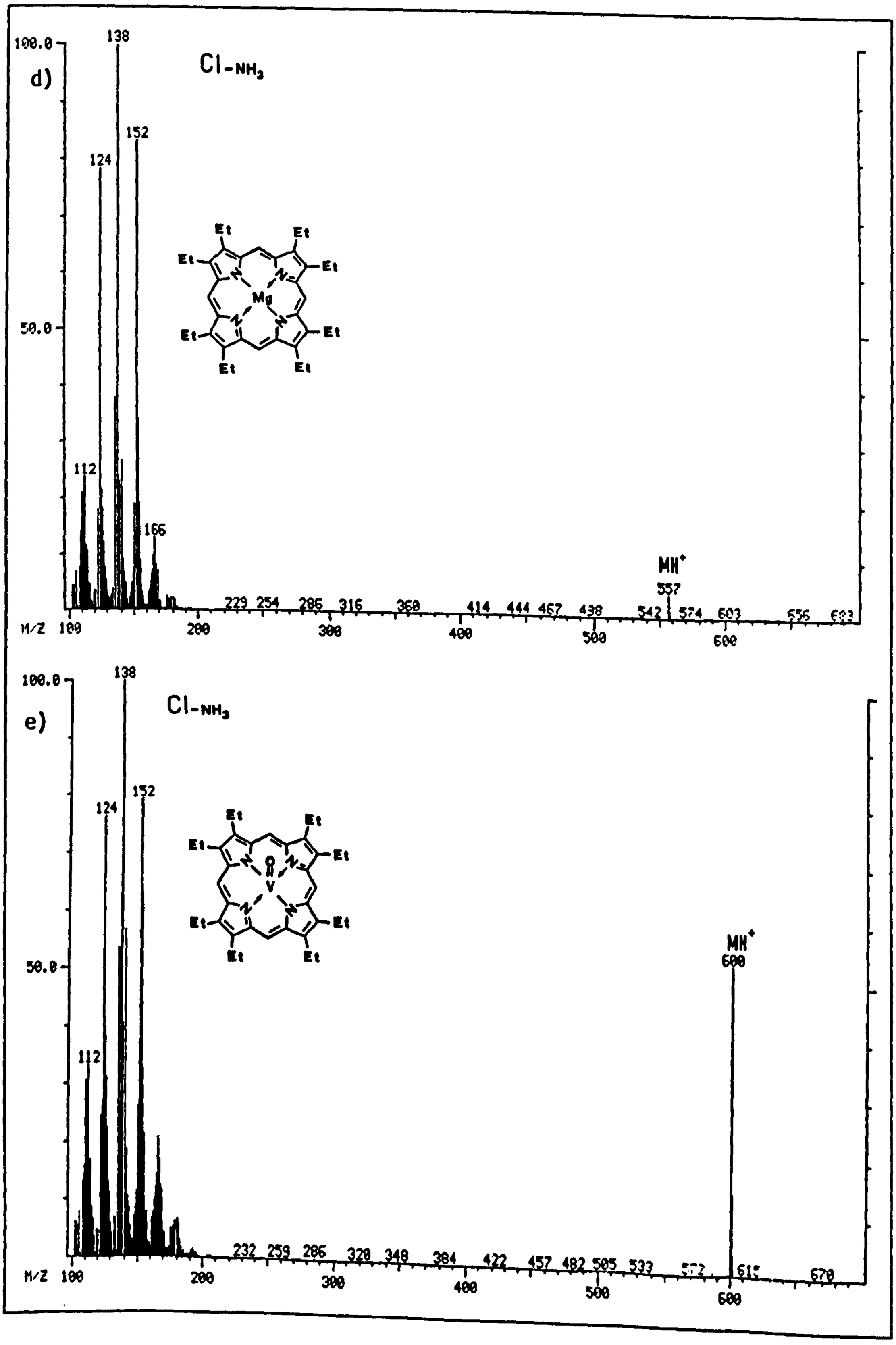
Fig.22 Molecular ion isotope pattern of certain metalloporphyrins, derived from the EI-mass spectra depicted in Fig.21.

IV.2.2.2. Chemical Ionisation Mass Spectrometry of Metalloporphyrins

Only a few investigations of the chemical ionisation mass spectra of metalloporphyrins have been reported so far (Evershed, R. P. et al., 1985; Tolf, B.R. et al.,1986) but from these published data it becomes evident that chemical ionisation mass spectrometry is less successful in structure elucidation of unknown metallated geoporphyrins than in the analysis of the respective demetallated compounds, the free bases. This is demonstrated in Fig. 23 showing six metallo-octaethylporphyrins bearing a number of chelating atoms of particular geochemical interest. The samples were introduced by capillary gas chromatography and ammonia was used as chemical ionisation reagent gas.




b)
Cl-NH3
100.0
50.0
124
138
152
112
166
Et
Co
MH+
592
204
238
261
286
330
363
398
439
491
519
542
606
645
670
M/Z
100
200
300
400
500
600
c)
Cl-NH3
124
138
152
110
166
Cu
MH+
596
219
246
273
307
351
385
457
511
612


d)
Cl-NH3
100.0
50.0
138
152
124
112
166
229 254 286 316 360 414 444 467 498 542 574 603 656 693
MH+
557
M/Z 100 200 300 400 500 600
Et
Mg
e)
Cl-NH3
100.0
50.0
138
152
124
112
232 259 286 320 348 384 422 457 482 505 533 572 615 670
MH+
600
M/Z 100 200 300 400 500 600
O
V

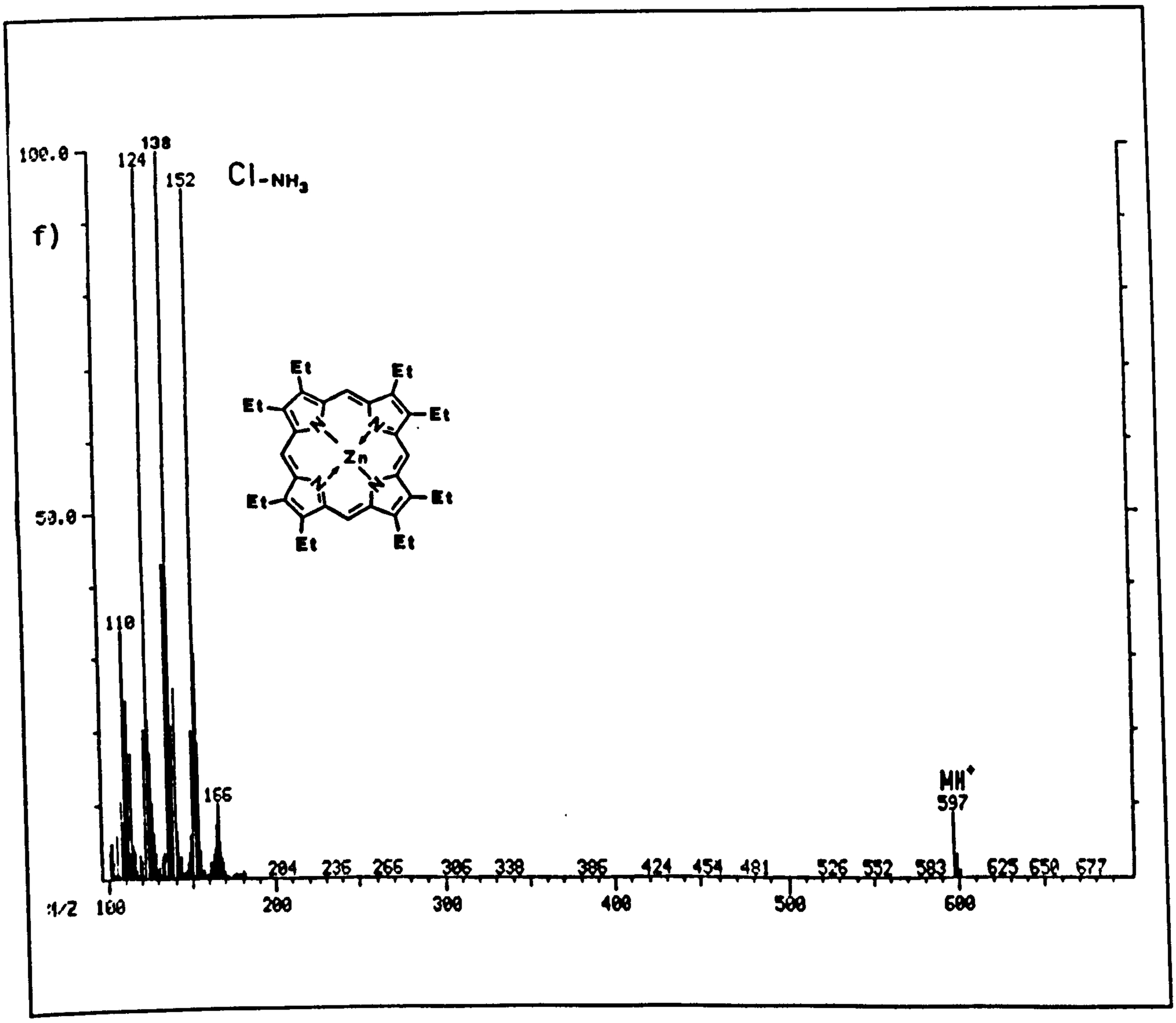


Fig.23 CI/NH_3-mass spectra of: a) Ni(II)octaethylporphyrin; b) Co(II)-octaethylporphyrin; c) Cu(II)octaethylporphyrin; d) Mg(II)octaethylporphyrin; e) V(IV)octaethylporphyrin; f) Zn(II)octaethylporphyrin.Introduction: capillary column. For experimental details see Section VII.1.3, 2.1 and 2.2.

In comparison to free-base octaethylporphyrin (see Fig 18c), the CI/NH_3 mass spectra of the respective metal chelates showed in the most cases a reduced abundance of the MH^+ quasi-molecular ions. Moreover, in all spectra none of the diagnostically important tri- and dipyrrole fragments could be detected. Instead, they gave a distinct fragmentation to the less informative monopyrrolic fragments at m/z 110, m/z 124, m/z 138 and m/z 152 (see Fig.20). But despite this limitation, structural information an be readily obtained from these data.

IV.2.4. Influence of the Ionisation Mode on the Chromatographic Separation Efficiency in GC/MS

Each chromatographic method, when combined with a mass spectrometer, can influence the resulting mass spectra to some extent, due to the influence of the respective mobile phase on the ionisation process. Vice versa, uncontrolled and in many cases unavoidable secondary chemical reactions in the ion source, particularly in chemical ionisation mode, can also influence the resolution of a GC/MS system. This is demonstrated in the GC/CIMS-chromatogram of octaethylporphyrin using methane as a reagent gas shown in Fig.24 below.

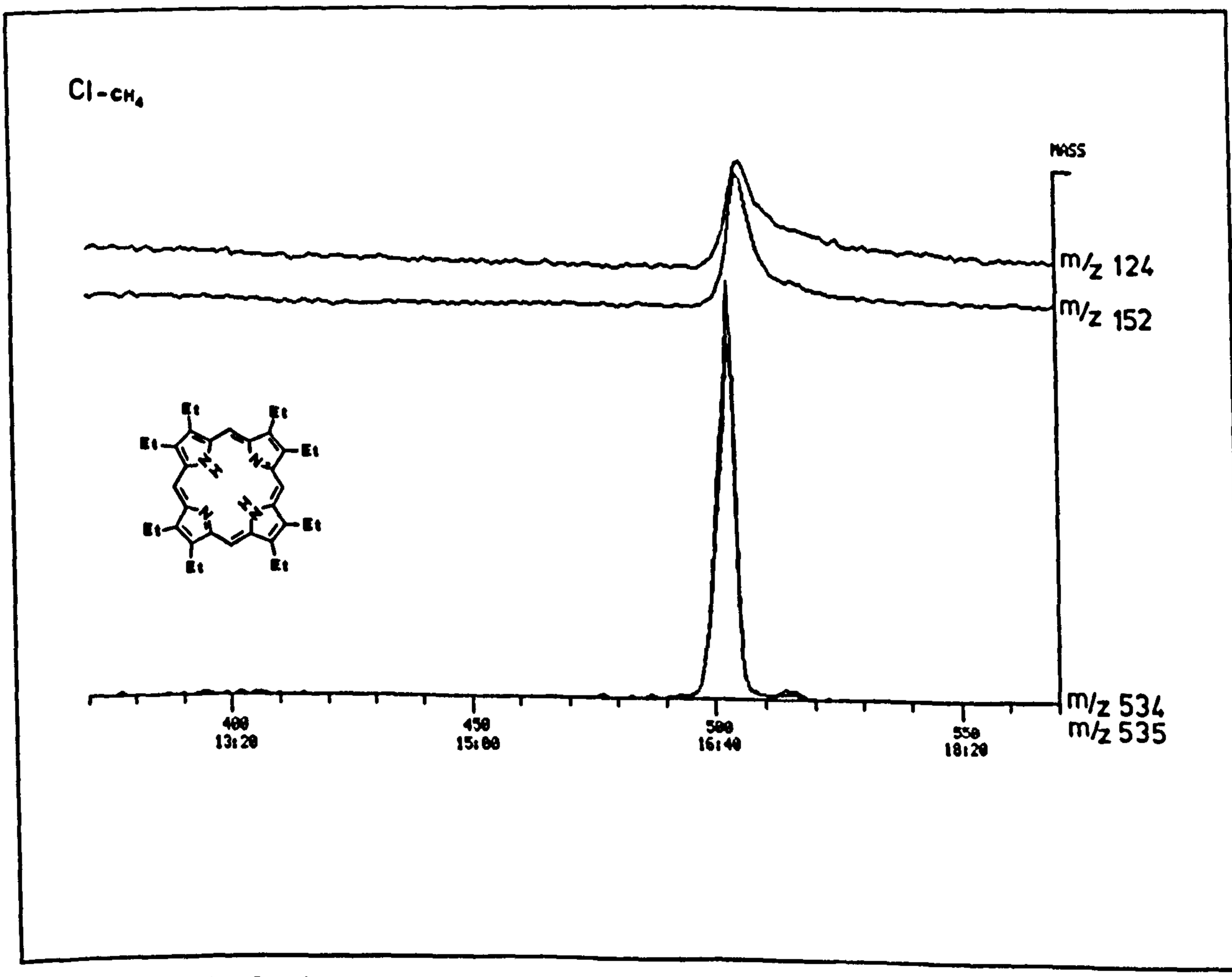
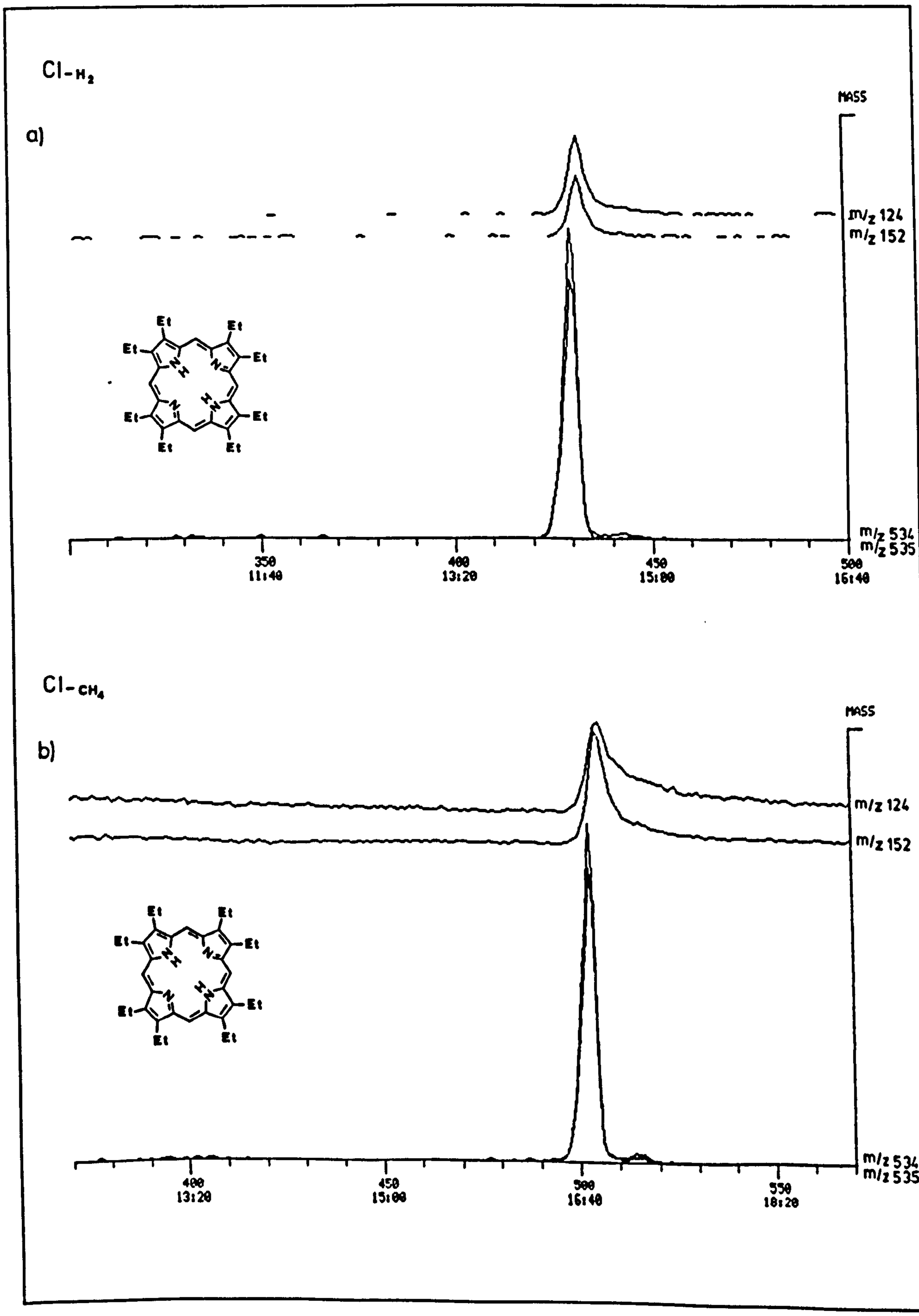


Fig.24 Single ion reconstructed gas chromatograms of octaethylporphyrin over m/z 124, m/z 152, m/z 534 and m/z 535. CI-gas: CH_4; Ion source temp.:180°C. See also Fig.18a.

Whereas both the total-ion-current chromatogram over a mass range m/z 100-700 (not depicted) and the two selected ion chromatograms over two monopyrrolic fragment ions at m/z 136 and m/z 152, exhibit broadened time profiles, the corresponding molecular-ion chromatograms over m/z 534 and m/z 535 show nearly perfect gaussian peaks. Since the molecular-ion profiles (molecular- and quasi-molecular ion) represent the passage of the GC-fraction through the ion source, the distorted peak shapes indicate that part of the sample remains in the ion volume after the chromatographic peak has passed. This phenomenon deteriorates the chromatographic resolution, comparable to an insufficient detector dead-volume and leads to a mix-up of consecutively eluting GC-fractions. It seems to depend on factors like reagent gas pressure, ion source temperature, state of contamination of the ion source and above all the extent of negative ion formation, and therefore of the nature of the reagent gas. Budzikiewicz interpreted the phenomenon as a reaction between sample molecules and CI-reagent gas prior to ionisation (Budzikiewicz, H., 1988). The influence of the CI-gas is of particular interest with respect to structure elucidation of geoporphyrin mixtures by means of GC/CIMS and is demonstrated in the following Figure 25 showing selected ion-chromatograms of octaethylporphyrin using hydrogen, methane and ammonia as reagent gases.


Cl-H2
a)
MASS
m/z 124
m/z 152
m/z 534
m/z 535
350
11:40
400
13:20
450
15:00
500
16:40
Et
NH
N
Cl-CH4
b)
MASS
m/z 124
m/z 152
m/z 534
m/z 535
400
13:20
450
15:00
500
16:40
550
18:20

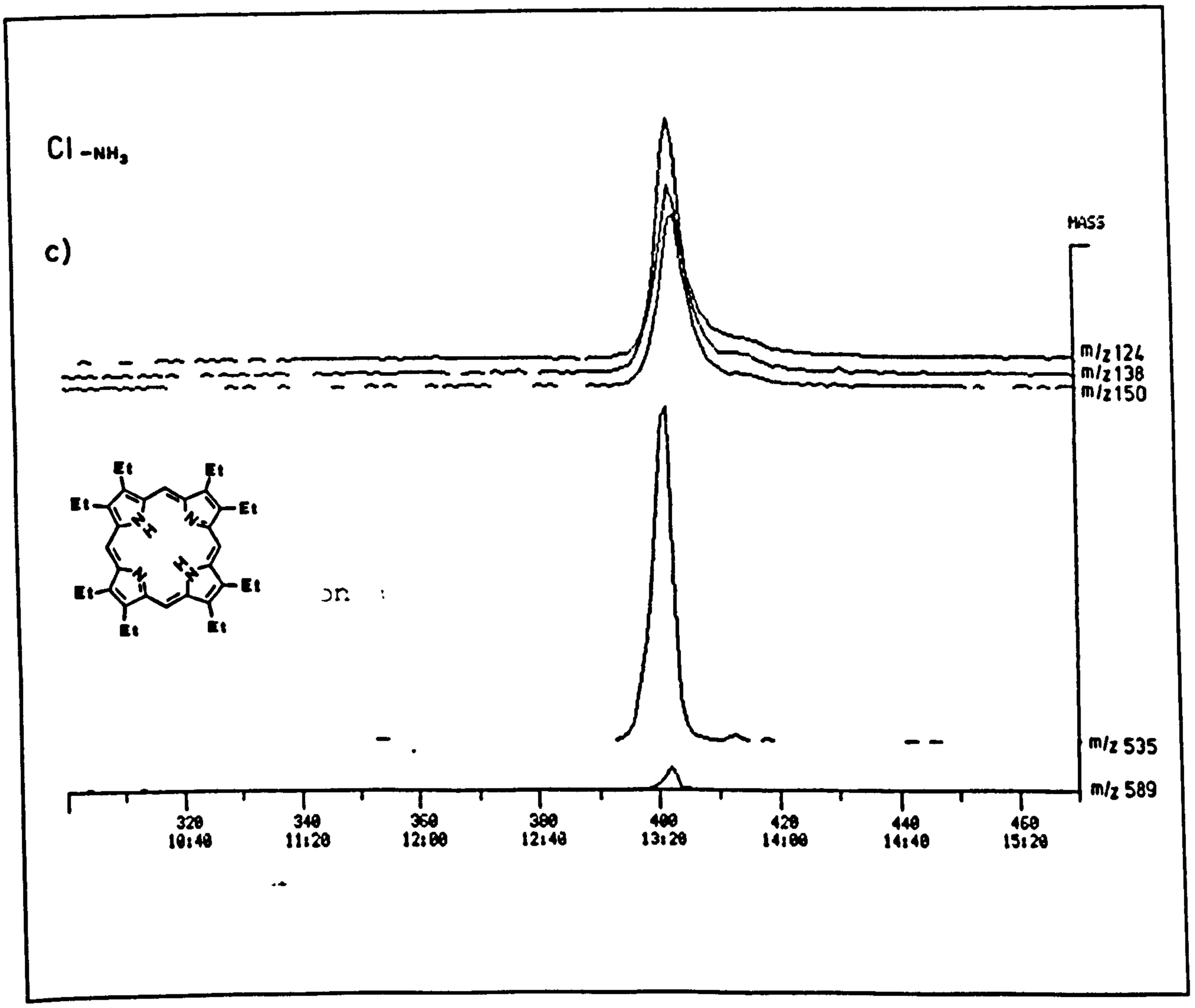


Fig.25 CI/NH_3 single ion reconstructed gas chromatograms of octaethylporphyrin over the monopyrrolic ions m/z 124, m/z 138, m/z 150 and the molecular- and quasi-molecular ions, respectively, at m/z 534, m/z 535 and m/z 589 $(M-2H+Fe+H)^+$. Reagent gas: a) H_2 b) CH_4 c) NH_3.

A kinetic model explaining the potential routes of these secondary reactions in the ion source was given by Sears et al.(Sears, L.J., 1987) for the production of negative ions. It is based on the assumption that beside gas-phase and surface-assisted free radical reaction the influence of ion-ion and ion-electron recombination as well as ion-wall neutralisation of negative charged molecular ions, leads to the above demonstrated phenomenon. The question whether this explanation holds true for positive ion formation as well, remains open at this time and can not be the subject of this work. However, from the selected ion chromatograms given in Fig.25 and 26 it can be concluded that on one hand the extent of secondary reactions is similar for both the free bases and the metalloporphyrins (Fig.26), and that on the other hand in respect of preservation of chromatographic efficiency, hydrogen and ammonia are the most suitable CI reagent gases for the mixture analysis of geoporphyrins. The most informative mass spectra are obtained by using ammonia and this reagent gas is therefore recommended for structure elucidation of unknown geoporphyrins by means of capillary GC/CIMS.

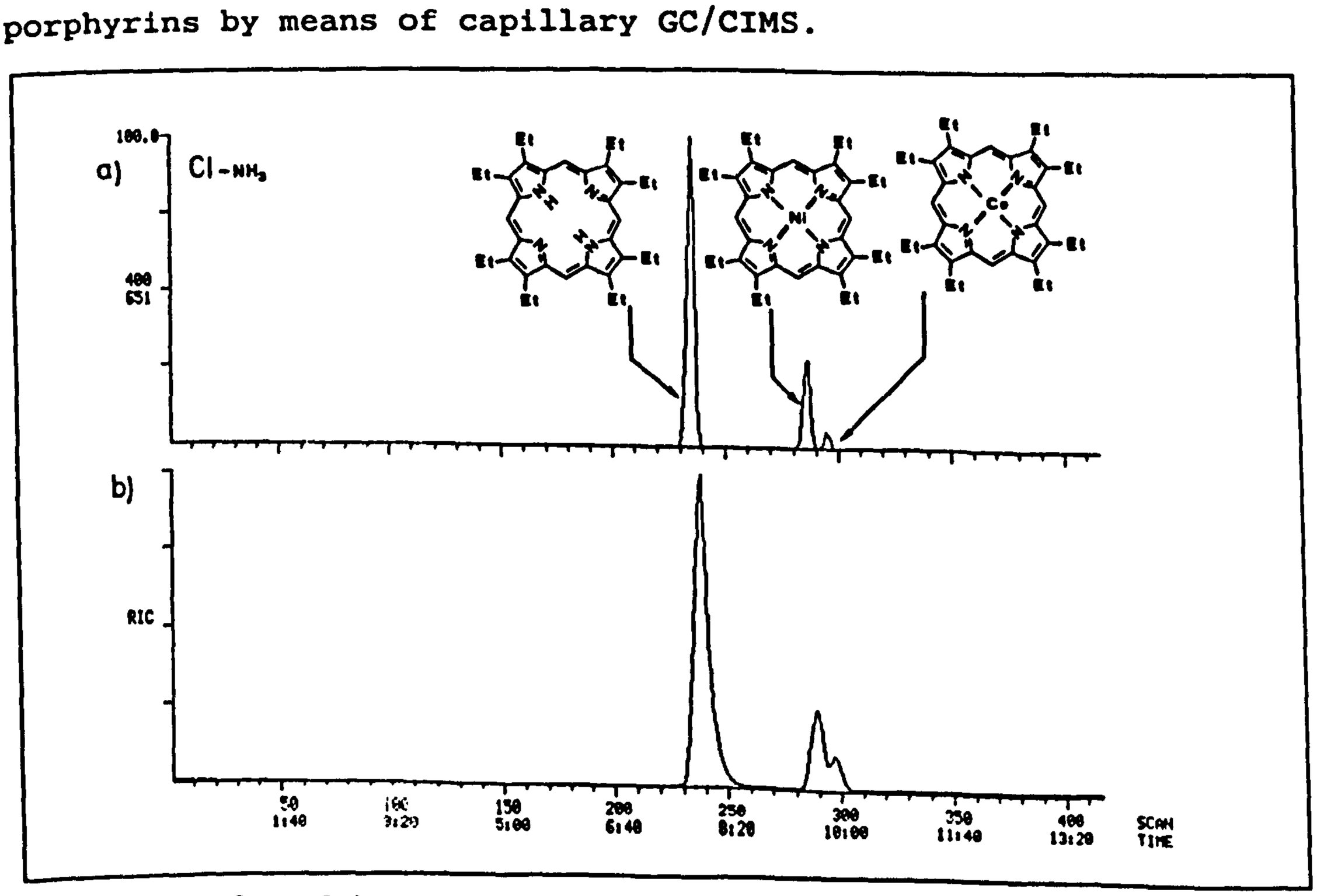


Fig.26 a) Selected ion current chromatogram over m/z 400-650; b) Total ion current chromatogram over m/z 100-700 of free-base-, nickel(II)- and cobalt(II)octaethylporphyrin.Reagent gas:NH_3. For experimental details see Section VII.2.1-2.2..

IV.2.4.1. Geoporphyrin Mass Spectra from GC/MS Runs.

In spite of optimised separation efficiency and the selectivity of the chosen high temperature capillary columns, in none of the application examples examined in the next chapter could petroporphyrins be separated as individual components. Each GC-peak contains a variety of homologues and isomers, and the characterisation of petroporphyrins by GC/MS has therefore to be carried out from mass spectra of mixtures. This is demonstrated in Fig.27.

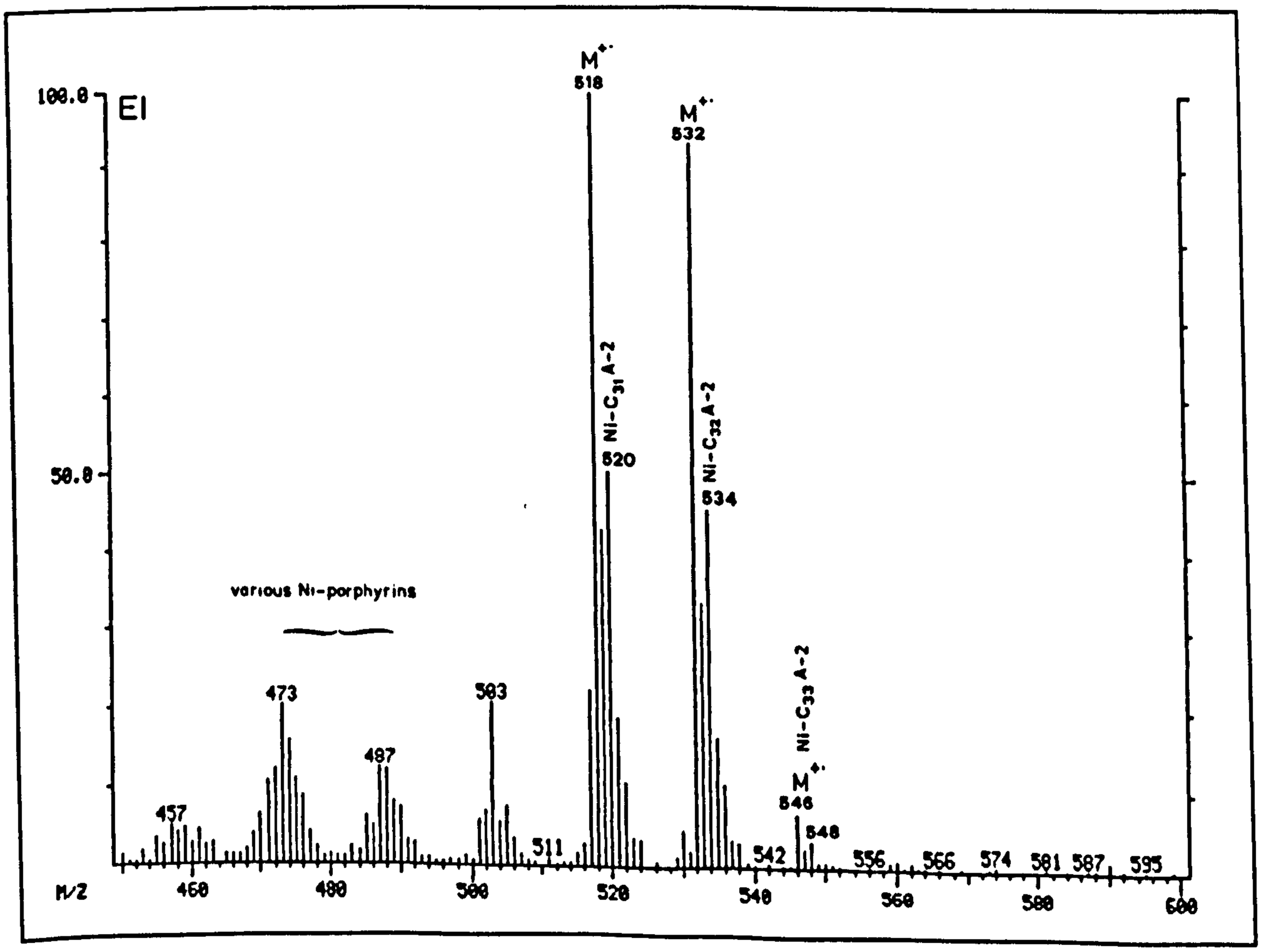


Fig.27 EI-mass spectrum of GC-peak P of a Julia Creek oil shale extract fraction JC2 (see Section V). The spectrum was derived from the TIC-chromatogram depicted in Fig.53.

The above mass spectrum is representative for the sort of spectra obtained from GC/MS analysis of geoporphyrins under normal conditions. It contains a cluster of molecular ions e.g. at m/z 546, m/z 532 and m/z 518, respectively, indicating a mixture of homologues. Nevertheless, due to the fact that the molecular ions in EI- spectra of petroporphyrins are dominant and subsequent fragmentation only of minor importance, the correlation of individual homologues as well as its semi-systematic characterisation is feasible even from these data.

In the case of CI-spectra, the unavoidable secondary reactions have to be considered as well. As discussed in the preceding section, they reduce the chromatographic resolution, and lead to an overlap of diagnostically important mono- and dipyrrolic fragments of subsequently eluting GC peaks. This makes it difficult to correlate the fragment ions with proper molecular ions, particularly on the back side of dominant GC-peaks. Thus, the CI-spectra derived from GC/MS runs of geoporphyrins, in particular of trace components, are only of limited significance in most cases.

The influence of the mobile phase on the reproducibility of CI-spectra has been already discussed in section III.2.1. For high temperature capillary gas chromatography the use of hydrogen as a carrier gas is an unavoidable prerequisite, and hydrogen clearly acts as a CI-reagent gas. Fortunately, hydrogen is next to ammonia the most suitable reagent gas(see the foregoing section). Since all the CI-spectra presented in this study are from GC/MS runs, it has to take into account that all indicated reagent gases contain about 20% of hydrogen (roughly estimated from ion source pressure) as a carrier gas introduced through the capillary column.

V. THE ANALYSIS OF OIL SHALE EXTRACTS

V.1. General

The objectives of the foregoing chapters were to discuss the analysis of geoporphyrins by means of high temperature capillary gas chromatography and capillary supercritical fluid chromatography in combination with atmospheric detectors and mass spectrometry in general in order to optimise the separation as well as the mass spectrometric ionisation methods for an investigation of the fractionated geoporphyrins.
In this chapter the optimised procedure was applied to the analysis of:

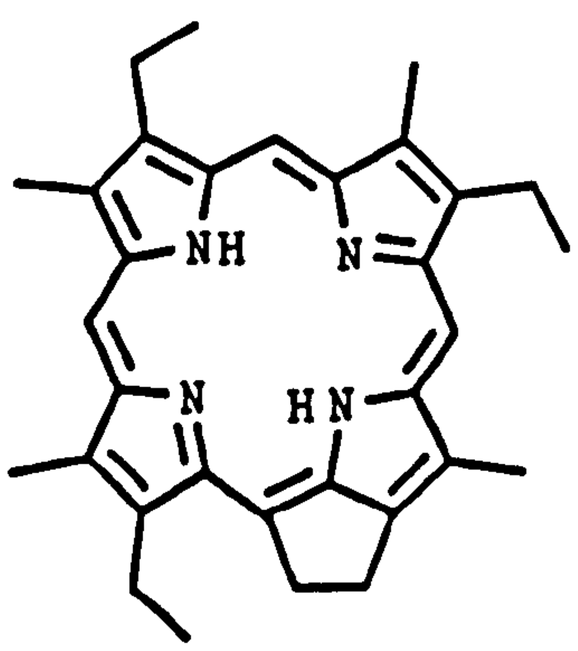
Julia Creek oil shale, Queensland, Australia, 144-165 mill. years old, Cretaceous

For this oil shale, direct analyses using capillary gas chromatography or supercritical fluid chromatography combined with mass spectrometry have not yet been reported to our knowledge.

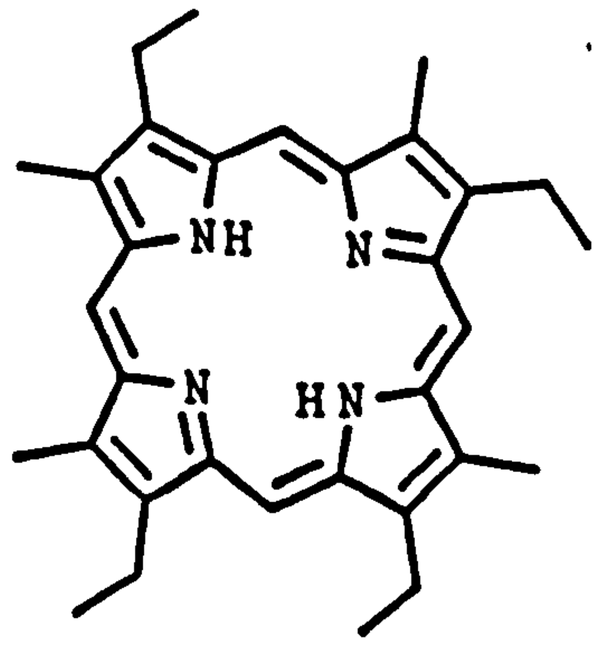
V.2. Julia Creek Oil Shale

Julia Creek oil shale is a carbonate deposit of Queensland, Australia and occurs in the Toolebuc formation present in the Eromanga Basin as well as in the southern part of Carpentaria Basin. The shales were formed during the Cretaceous period (ca. 144-165 mill. years ago). They have been deposited in a marine environment under reducing conditions in sheltered areas of a shallow epicontinental sea (Exon and Senior, 1976).
The organic matter is of algal origin but also contains residues of land plants.
Julia Creek oil shales were recently investigated by several Australian groups by means of HPLC separation and subsequent NMR and MS examination (Fookes, C.J.R, 1983a, 1983b, Fookes et al. 1983 and Ekstrom et al., 1983). In these papers the porphyrin structures listed below have been elucidated either completely or partially.

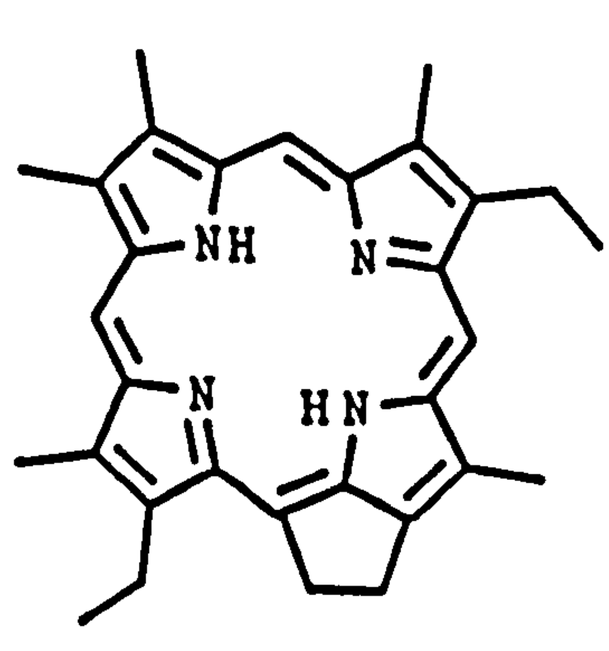
TABLE 1 Elucidated Geoporphyrin Structures of Julia Creek Oil Shale



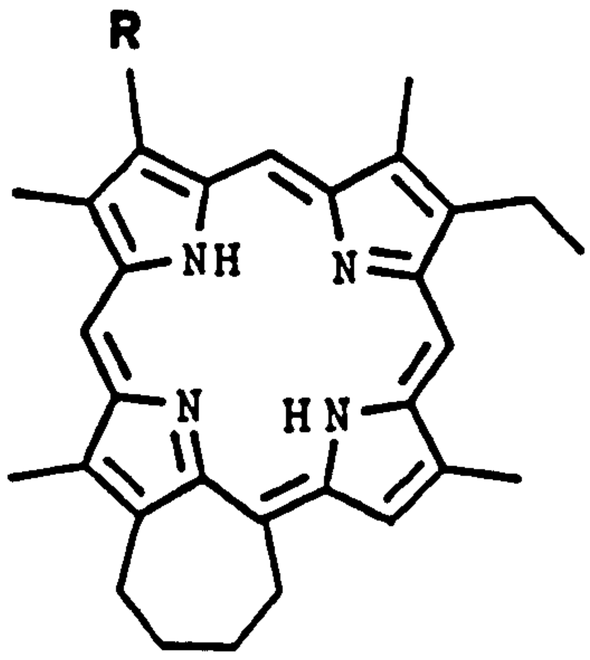
13,15-Ethano-3,8,17-triethyl-2,7,12,18-tetramethylporphyrin



2,7,12,18-Tetramethyl-3,8,13,17-tetraethylporphyrin



13,15-Ethano-8,17-diethyl-2,3,7,12,18-pentamethylporphyrin



20 $R = C_2H_5$ *15,17-Butano-3,8-diethyl-2,7,12,18-tetramethylporphyrin*

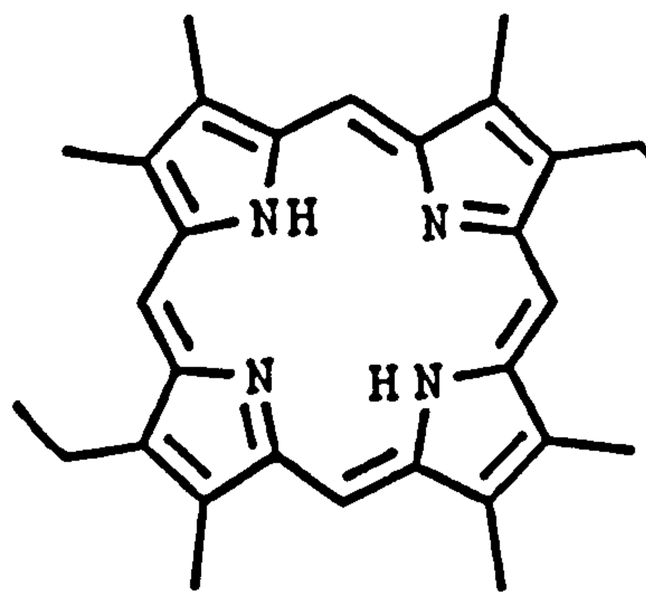
21 $R = CH_3$ *15,17-Butano-8-ethyl-2,3,7,12,18-pentamethylporphyrin*

22 $R = H$ *15,17-Butano-8-ethyl-2,7,12,18-tetramethylporphyrin*

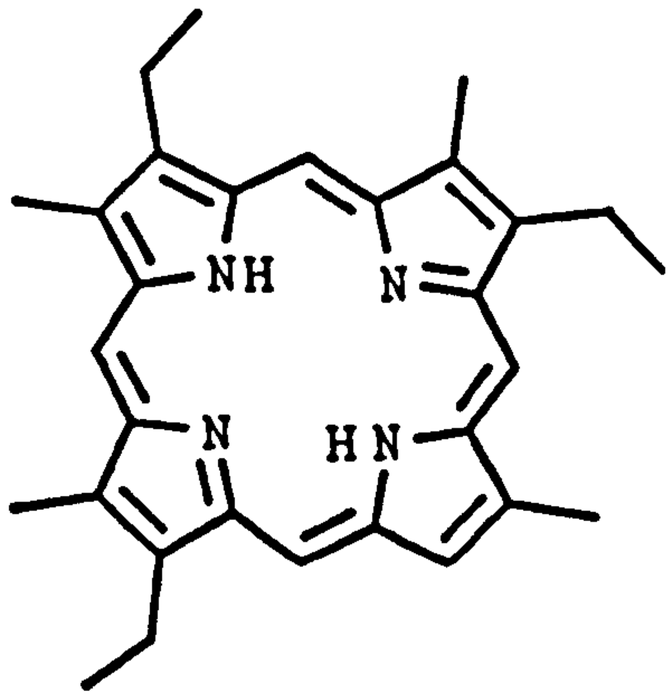
R = C_2H_5

R = CH_3

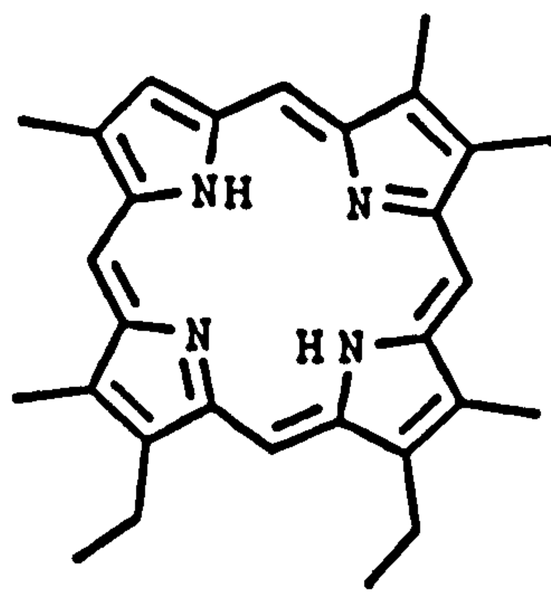
R = H



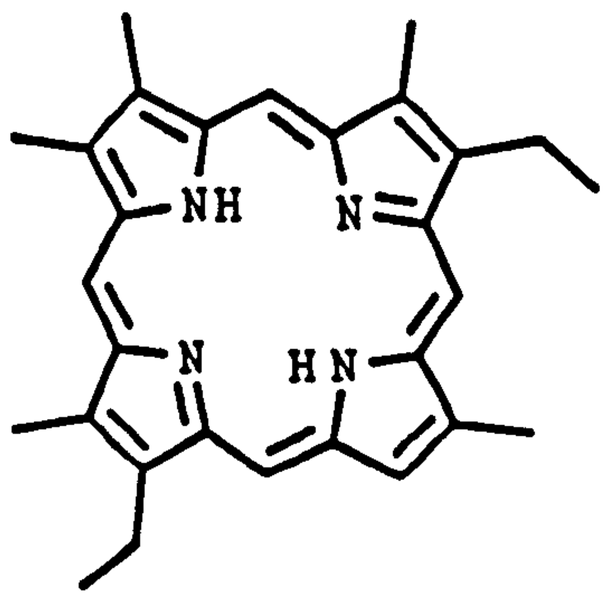
8,18-Diethyl-2,3,7,12,13,17-hexamethylporphyrin



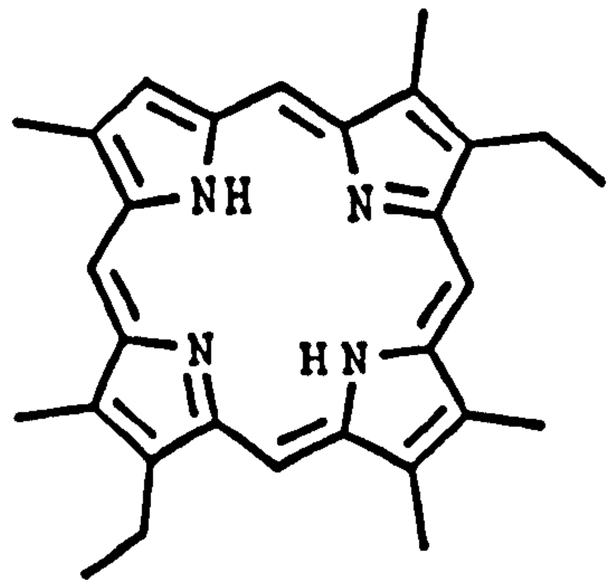
3,8,17-Triethyl-2,7,12,18-tetramethylporphyrin



8,13,17-Triethyl-2,7,12,18-tetramethylporphyrin



8,17-Diethyl-2,3,7,12,18-pentamethylporphyrin



8,17-Diethyl-2,7,12,13,18-pentamethylporphyrin

V.2.1. The Analysis of Julia Creek Oil Shale

V.2.1.1. The First Overview

The extraction of the pulverised Julia Creek oil shale was carried out with 150ml chloroform for 12h in a Soxhlet extractor as described in Section VII.1.4. The deep violet solution was reduced to a volume of 10ml by rotary evaporation. An aliquot of 10ul was made up to a volume of 50ul and taken for a first overview analysis by capillary-GC using FID detection.

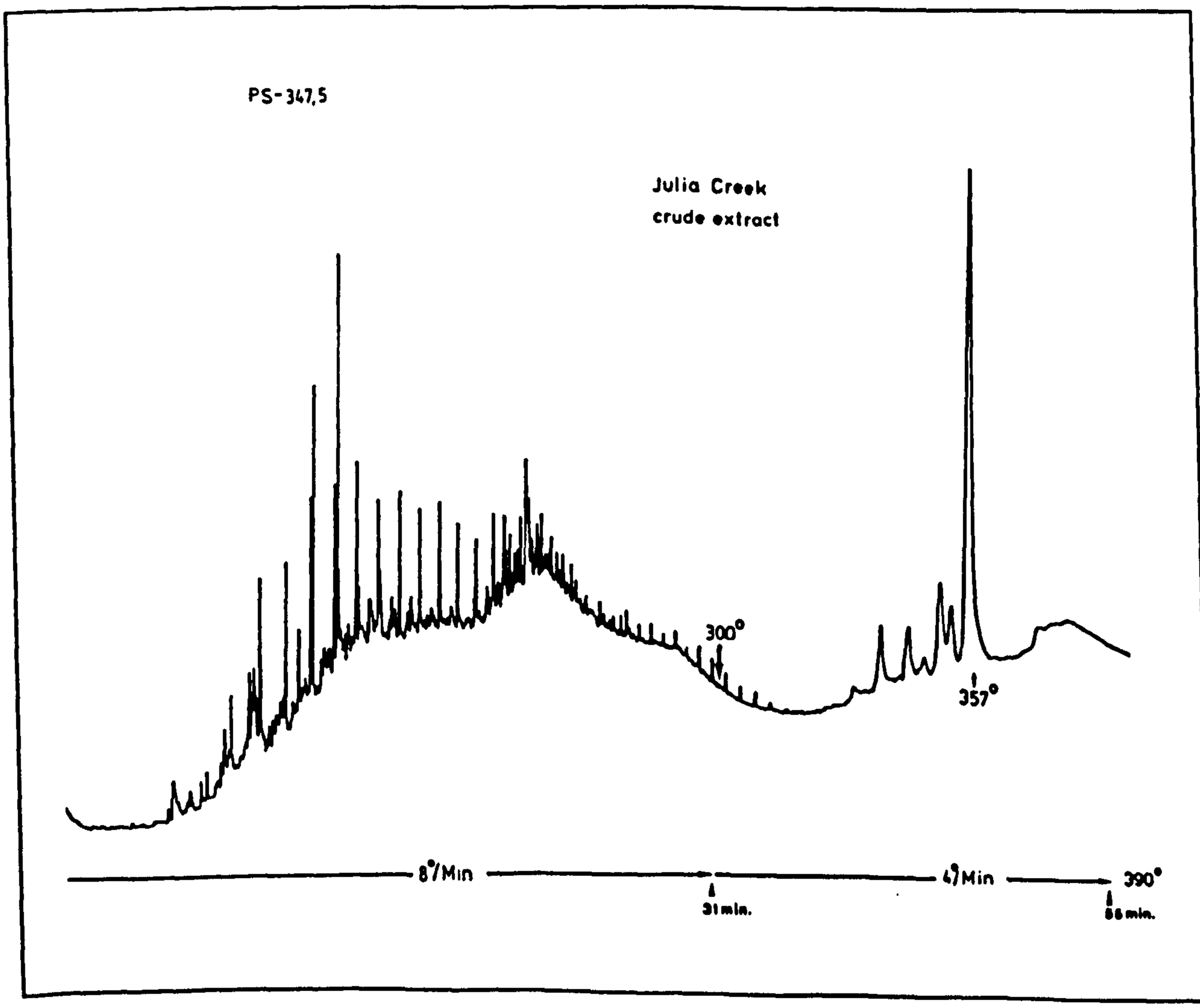


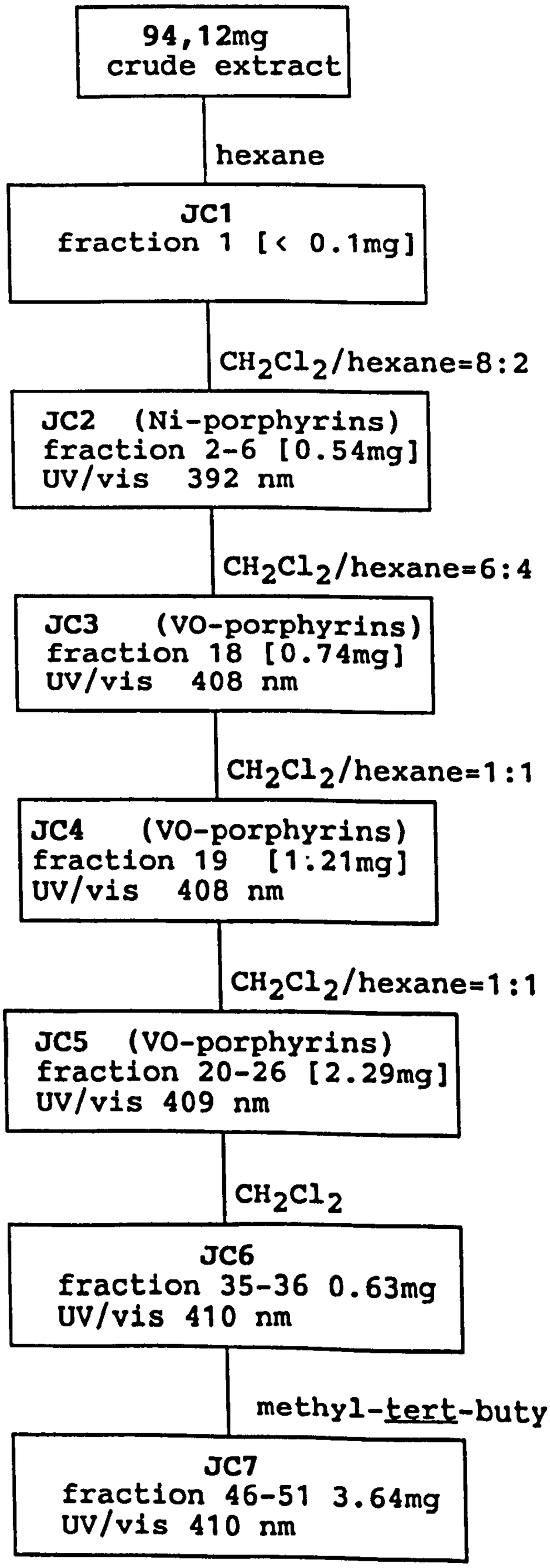
Fig.28 FID chromatogram of a crude chloroform extract of Julia Creek oil shale. Column: 20m x 0.3mm, coated with PS-347,5.

Since the crude extract contains significant amounts of nonvolatile organic residues, the entrance of the capillary column was irreversibly contaminated after several on-column injections, and covered with dark spots. As shown in Fig.28 the residue obviously postponed the evaporation of the high boiling components and affected the peak shape and response, particularly of adsorptive components.

It was the original aim to analyse the crude extracts of the oil shales directly (Blum W. et al., 1988a), because we did not want to risk the loss of trace components by pre-gas chromatographic clean-up, demetallation, silicon insertion and derivatisation etc.. But for several reasons we had to leave this avenue. A liquid chromatographic pre-separation step turned out to be an inescapable prerequisite, on one hand in order to protect the capillary columns and to overcome the serious problem of column contamination and on the other hand to improve the separation of metalloporphyrins and to master the complexity of the oil shale extracts to a greater extent.

For this purpose, the crude extract was fractionated into sixty portions by means of medium pressure column liquid chromatography over silica gel. This way, one gets rid of the chloroform soluble but involatile constituents from the bulk of the sample. The fractions with same UV absorption were combined, so that six main-fractions remained(JC2 - JC7), from which JC2-JC5 could be further examined by GC and GC/MS, respectively. The separation scheme employed in the fractionation of Julia Creek oil shale crude extract is depicted below. Details of the liquid chromatographic method are given in Section VII.2.5.

Table 2


94,12mg
crude extract
hexane
JC1
fraction 1 [< 0.1mg]
CH_2Cl_2/hexane=8:2
JC2 (Ni-porphyrins)
fraction 2-6 [0.54mg]
UV/vis 392 nm
CH_2Cl_2/hexane=6:4
JC3 (VO-porphyrins)
fraction 18 [0.74mg]
UV/vis 408 nm
CH_2Cl_2/hexane=1:1
JC4 (VO-porphyrins)
fraction 19 [1:21mg]
UV/vis 408 nm
CH_2Cl_2/hexane=1:1
JC5 (VO-porphyrins)
fraction 20-26 [2.29mg]
UV/vis 409 nm
CH_2Cl_2
JC6
fraction 35-36 0.63mg
UV/vis 410 nm
methyl-tert-butyl-ether
JC7
fraction 46-51 3.64mg
UV/vis 410 nm

V.2.1.2 Characterisation of the Individual Liquid Chromatographic Fractions

The analysis of the hydrocarbons, as obtained in fraction JC1 (see Fig.29) is not the subject of this work. Thus, the analysis by GC/MS is concentrated on the coloured, gas chromatographable fractions JC2-JC5. The respective FID overview chromatograms on an apolar, PS-347.5 coated column are shown in Figs.30-36.

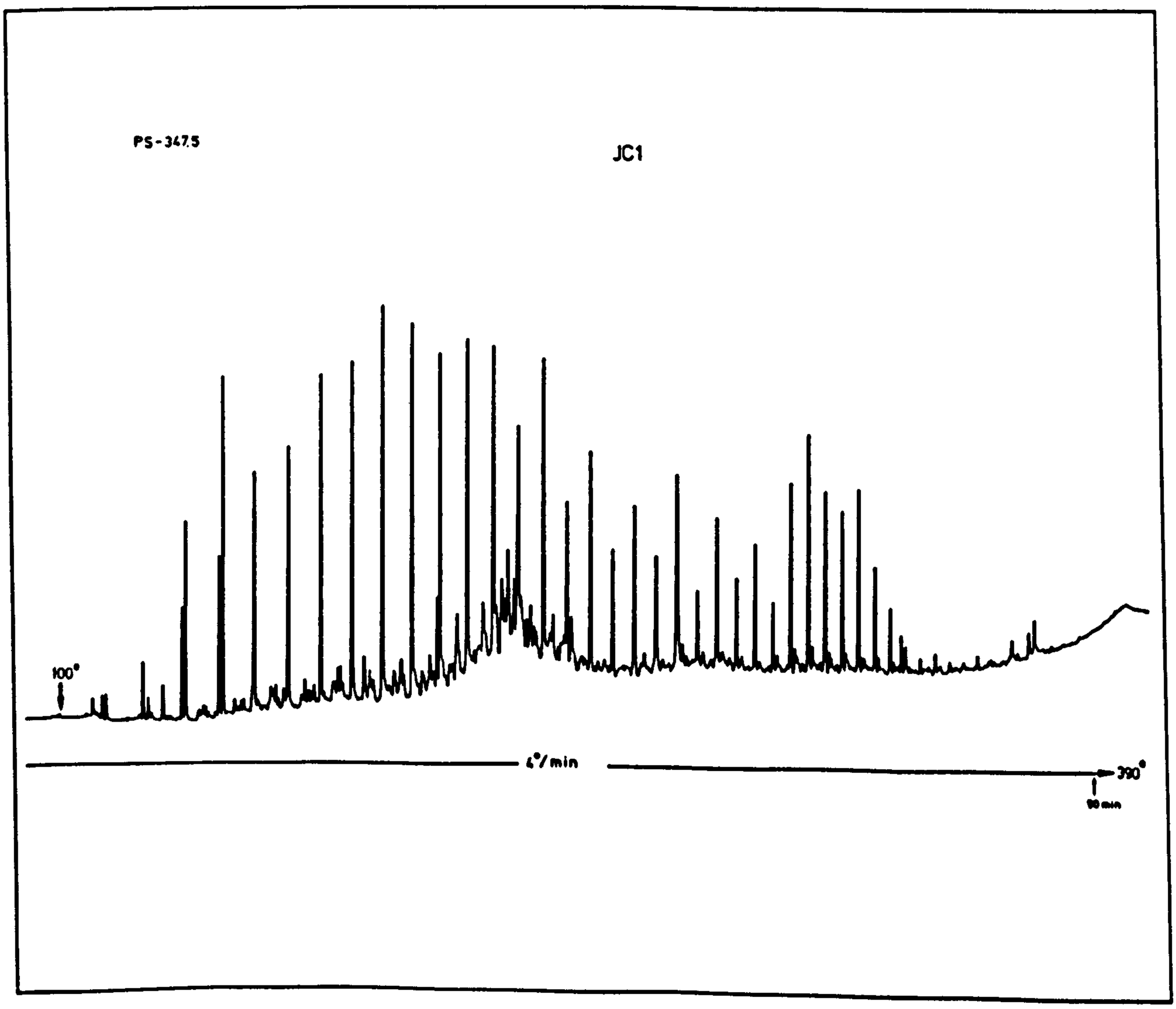


Fig.29 FID chromatogram of fraction JC1 of Julia Creek oil shale extract. Column: PS-347.5, 20m x 0.3mm.

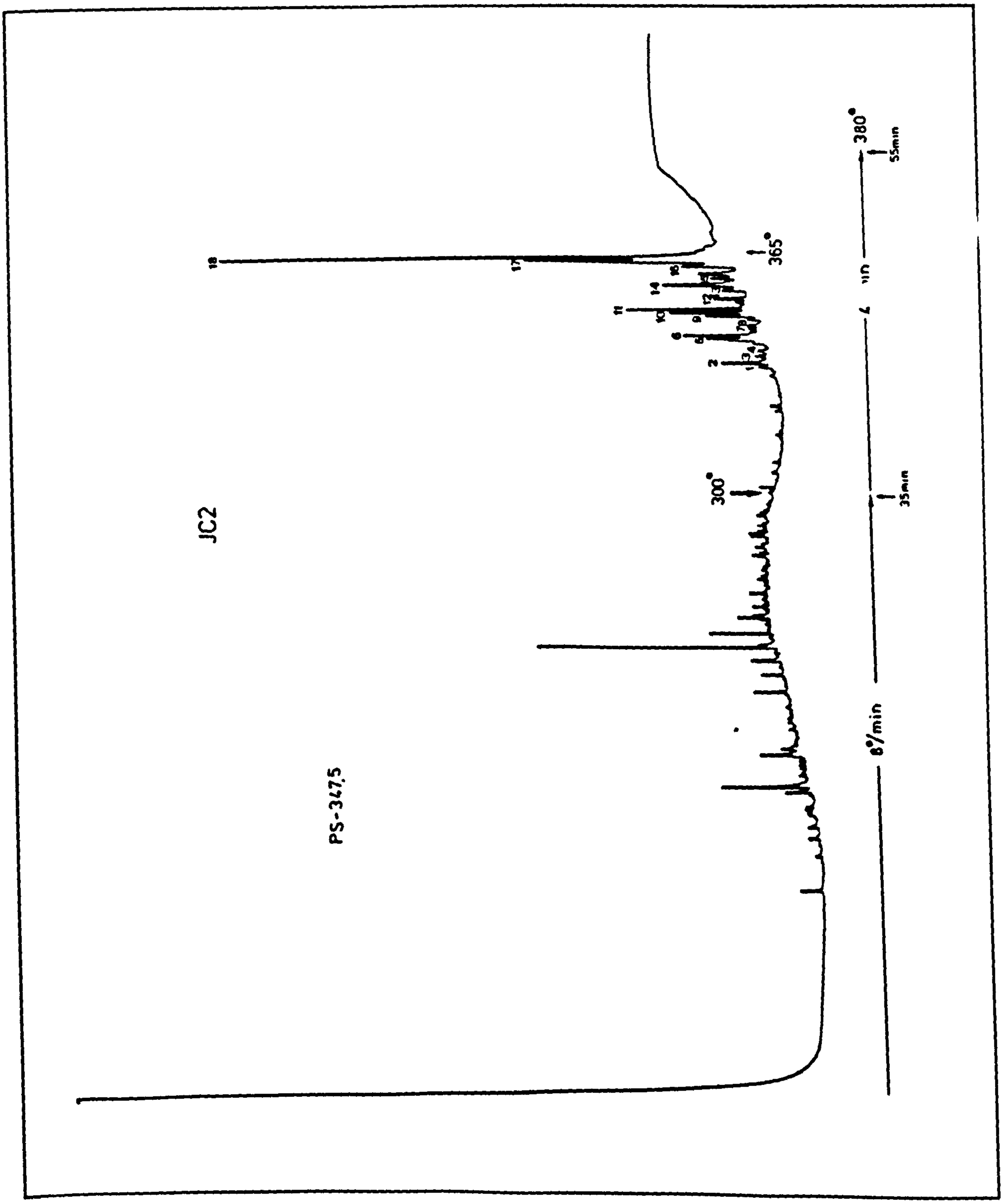


Fig.30 FID chromatogram of fraction JC2 of Julia Creek oil shale extract.
Column: PS-347.5, 20m x 0.3mm.

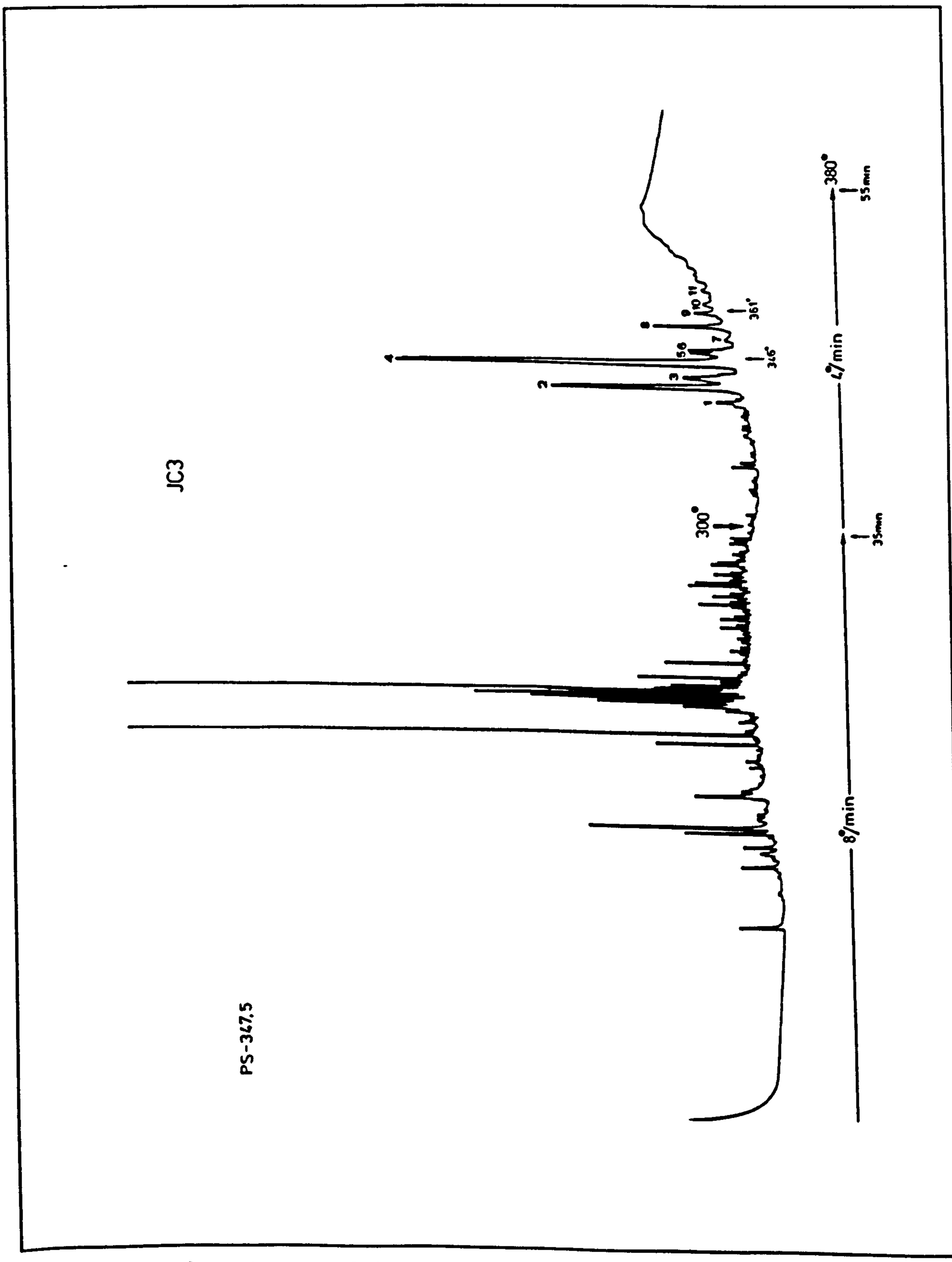


Fig.31 FID chromatogram of fraction JC3 of Julia Creek Oil shale extract. Column: PS-347.5, 20m x 0.3mm.

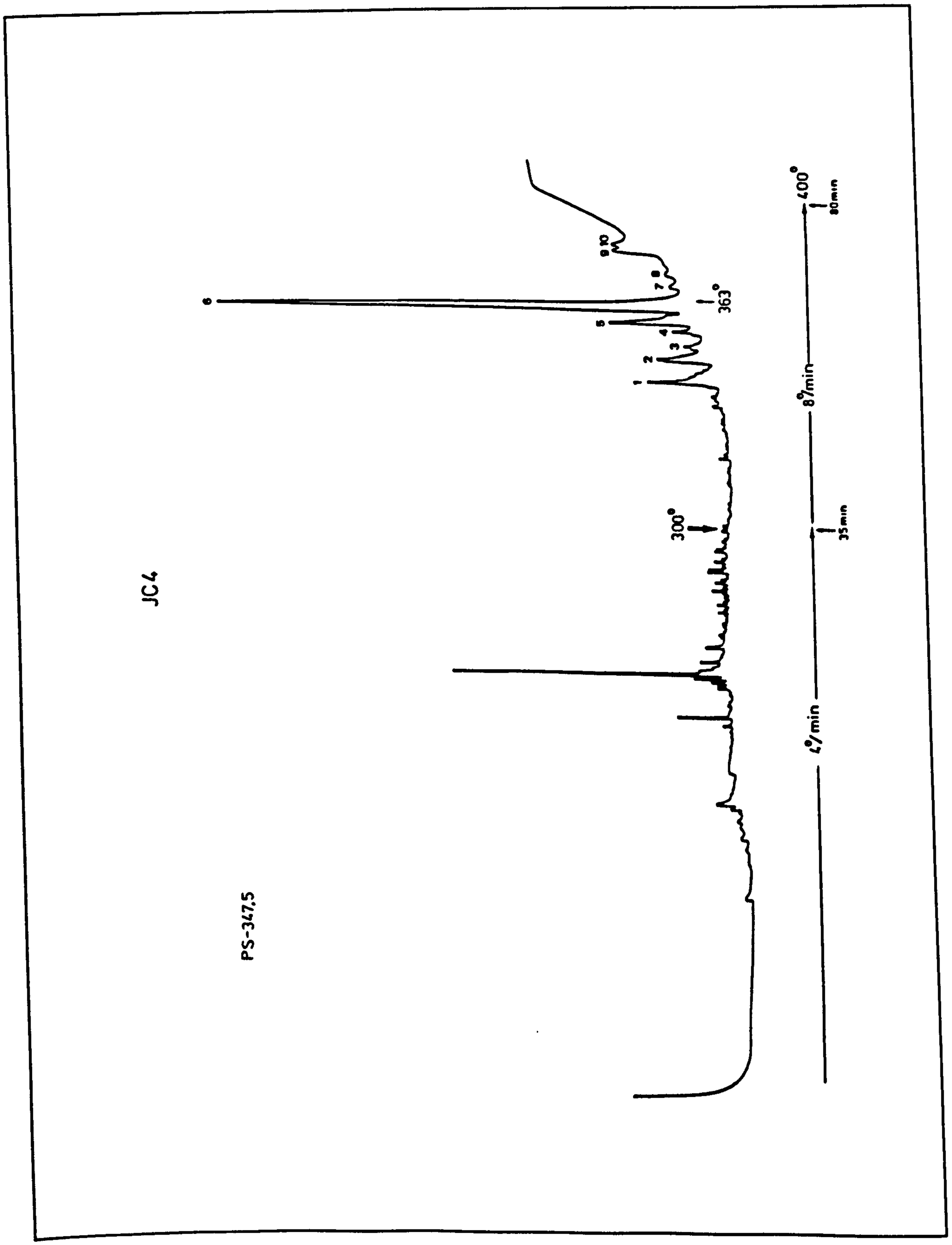


Fig.32 FID chromatogram of fraction JC4 of Julia Creek oil shale extract. Column: PS-347.5, 20m x 0.3mm.

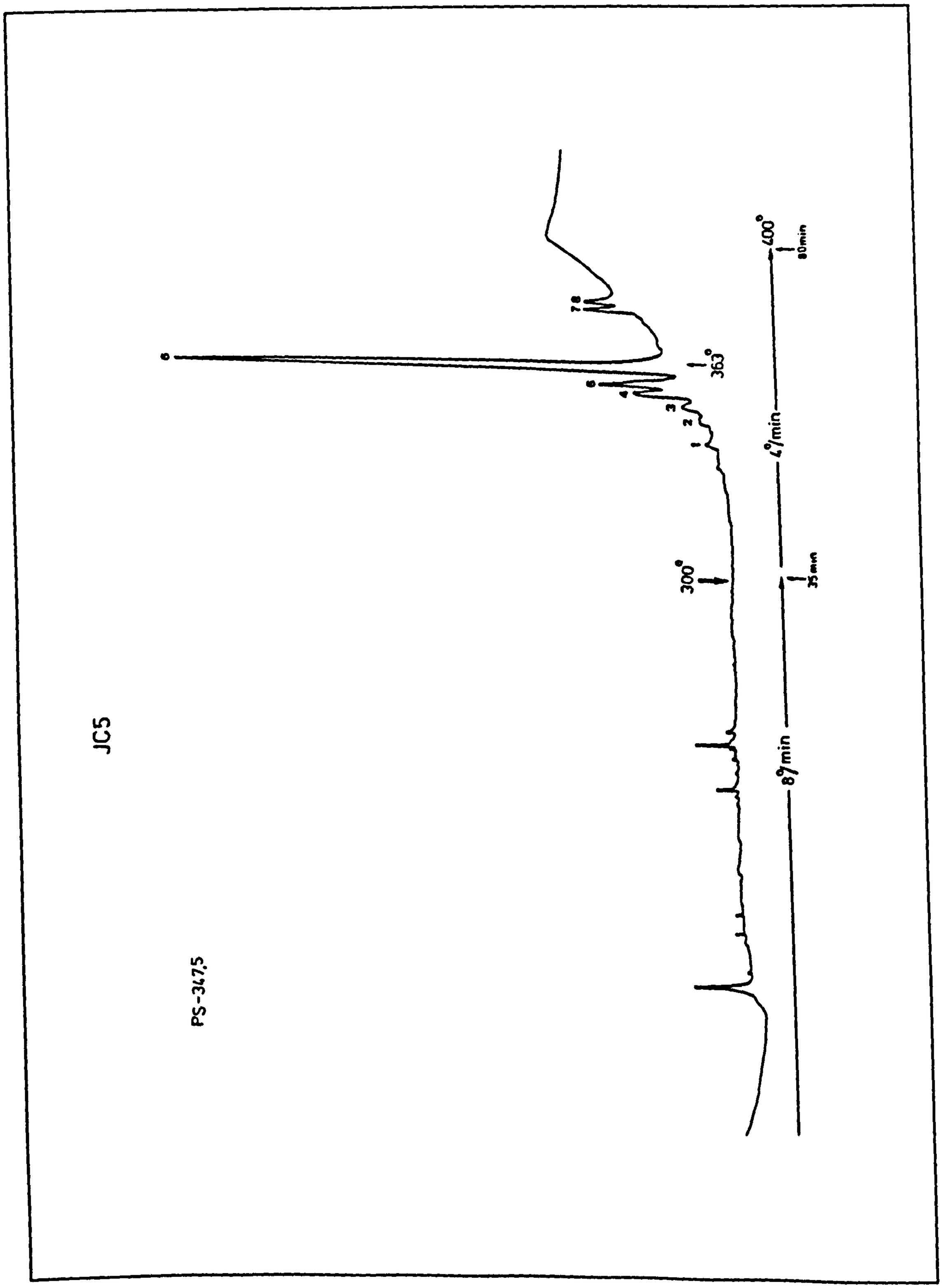


Fig.33 FID chromatogram of fraction JC5 of Julia Creek oil shale extract. Column: PS-347.5., 20m x 0.3mm.

V.2.1.3. Semi-Systematic Characterisation of the Metallated Geoporphyrins in Fraction JC2, JC3 and JC5

V.2.1.3.1. Fraction JC2

V.2.1.3.1.1. GC/MS Analysis of JC5 on a PS-090 (20% Phenyl) Coated Column

It is advisable to start the investigation of unknown petroporphyrins by gas chromatography on apolar coated columns, because of the lowest possible retention temperatures and the highest possible application range. But as already discussed in Section I.3.2.2., for many petroporphyrin extracts, an improved resolution was achieved on medium polar columns. Therefore, the examination was continued on a PS-090 coated capillary.

GC/MS analyses were performed on a Finnigan 4600 mass spectrometer, linked by the interface described in Section III. 1. to a Carlo Erba 4160 gas chromatograph, equipped with a constant pressure/constant flow regulator. In order to increase the sensitivity, and because the EI spectra of metalloporphyrins exhibit only negligible amounts of ions in the lower mass range, a selective scan range between m/z 400-700 was chosen.

The FID- and the EI total ion current (TIC) chromatograms of the first petroporphyrin fraction JC2 are shown in Fig.35a-b. They are representative for the transfer of FID results to high temperature GC/MS. At first glance, both chromatograms look similar. But, a closer look reveals that not all of the low concentration compounds indicated on the FID-trace could be recorded on the MS output as well, though the same capillary column was used. Moreover, the chromatographic resolution of the TIC chromatogram is somewhat reduced and some of the trace components disappear probably because they are absorbed on the (well deactivated) interface line (see Section VII.2.3.1.).

However, about 12 of the most important peaks observed on the FID record were transferred into the ion source of the mass spectrometer and their mole masses gave the basis for a semi-systematic assignment.

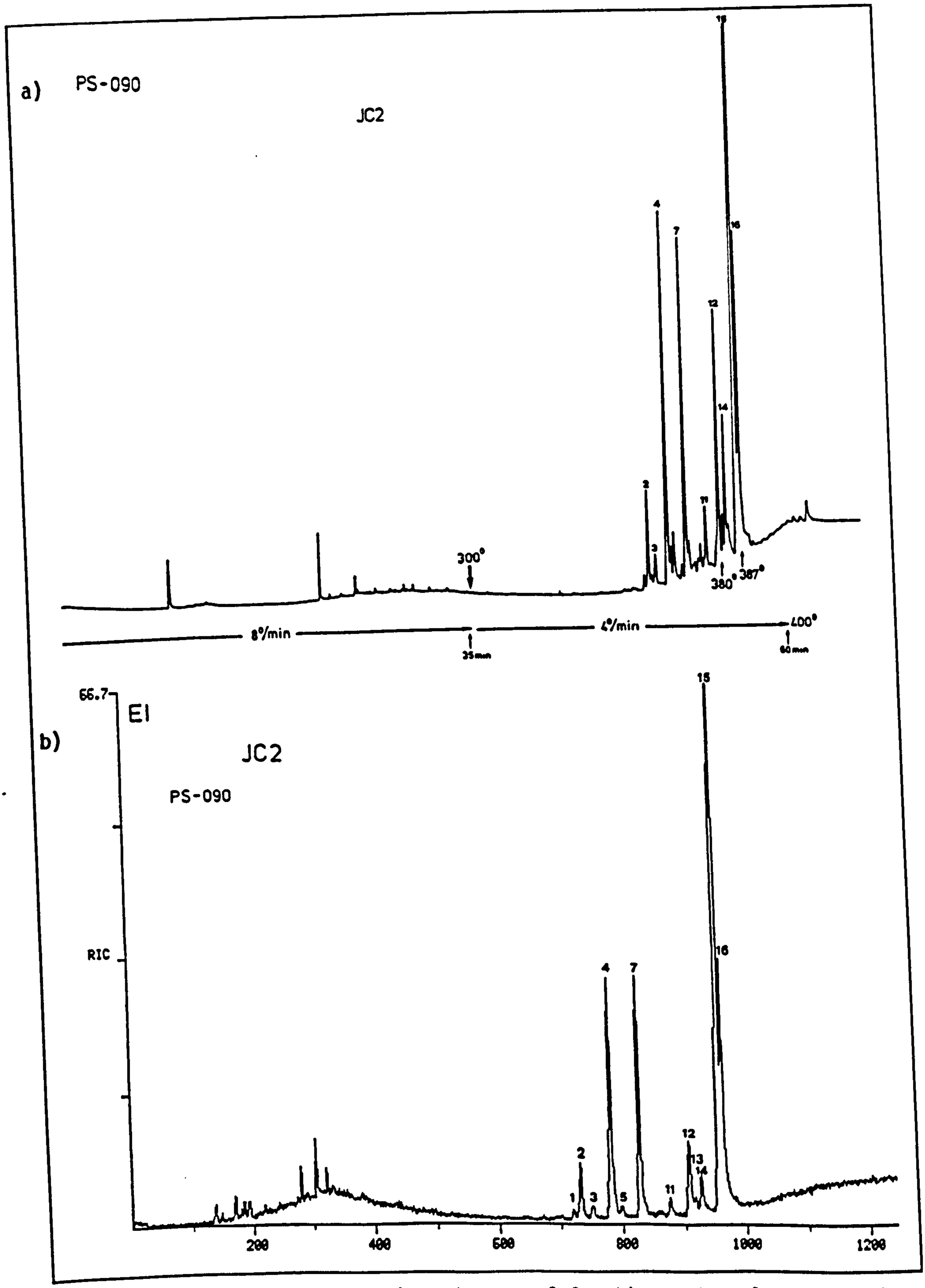


Fig.35 a) FID-, b) EI TIC-chromatogram of fraction JC2. Column: PS-090, 20m x 0.3mm. Scan range: m/z 400-700, cycle time 2 sec.

The total amount of the ions in the mass range of 400-700 covered by the GC-peaks 1, 2, 3, 4, 5, 7, 11, 12, 13, 14, 15 and 16 is shown in Fig.36-46. The summed spectrum reveals on one hand that each peak contains a variety of even and odd numbered (molecular?) ions, and on the other hand the difficulties to correlate particular ions to certain metalloporphyrins, especially if the ion current signals are of low intensities.

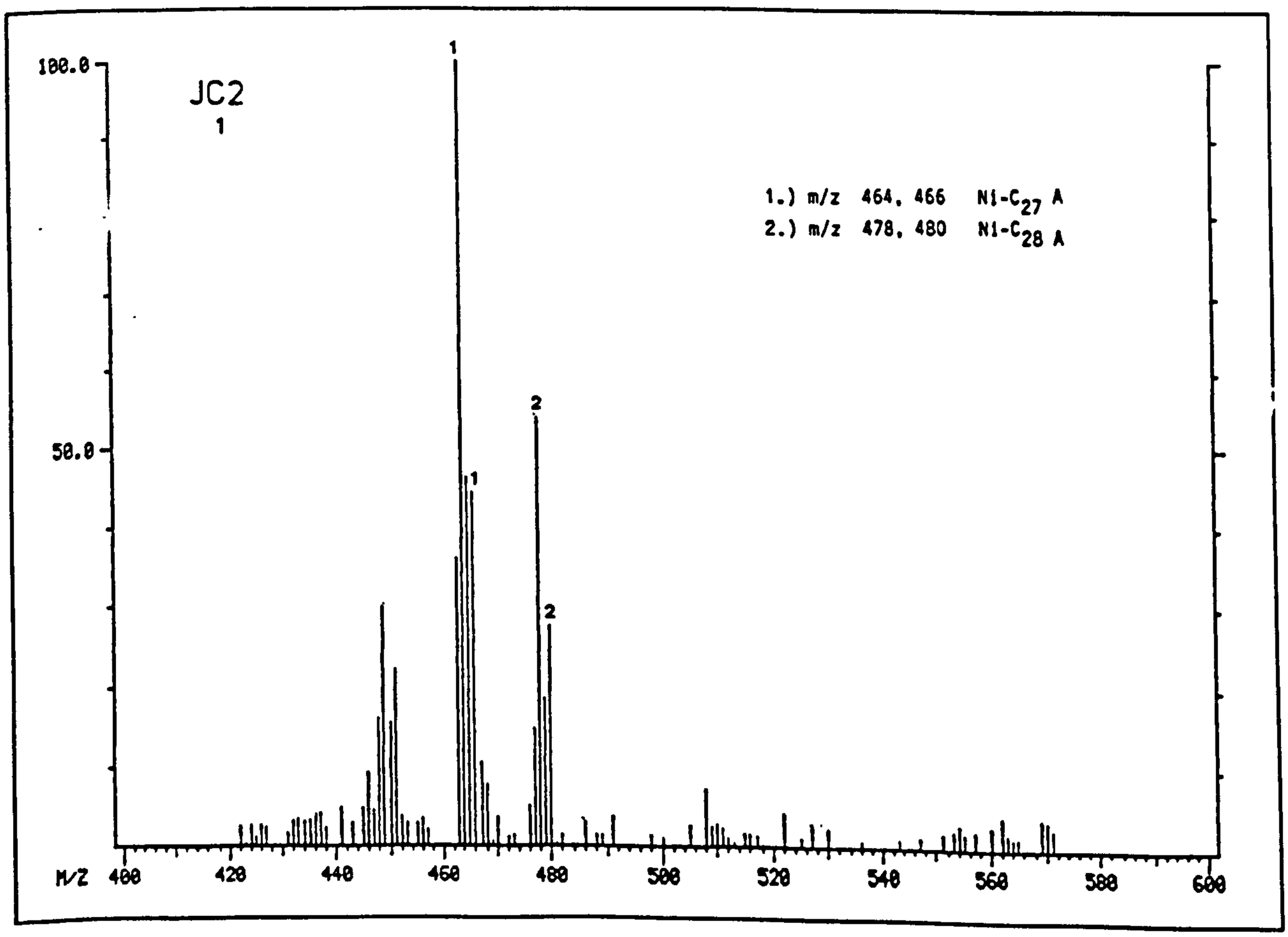


Fig.36 Summed mass spectrum of scans 719-730 (peak 1) from TIC chromatogram of JC2 shown in Fig.35. (The nature of the odd numbered ions e.g. at m/z 463 is explained in the text.)

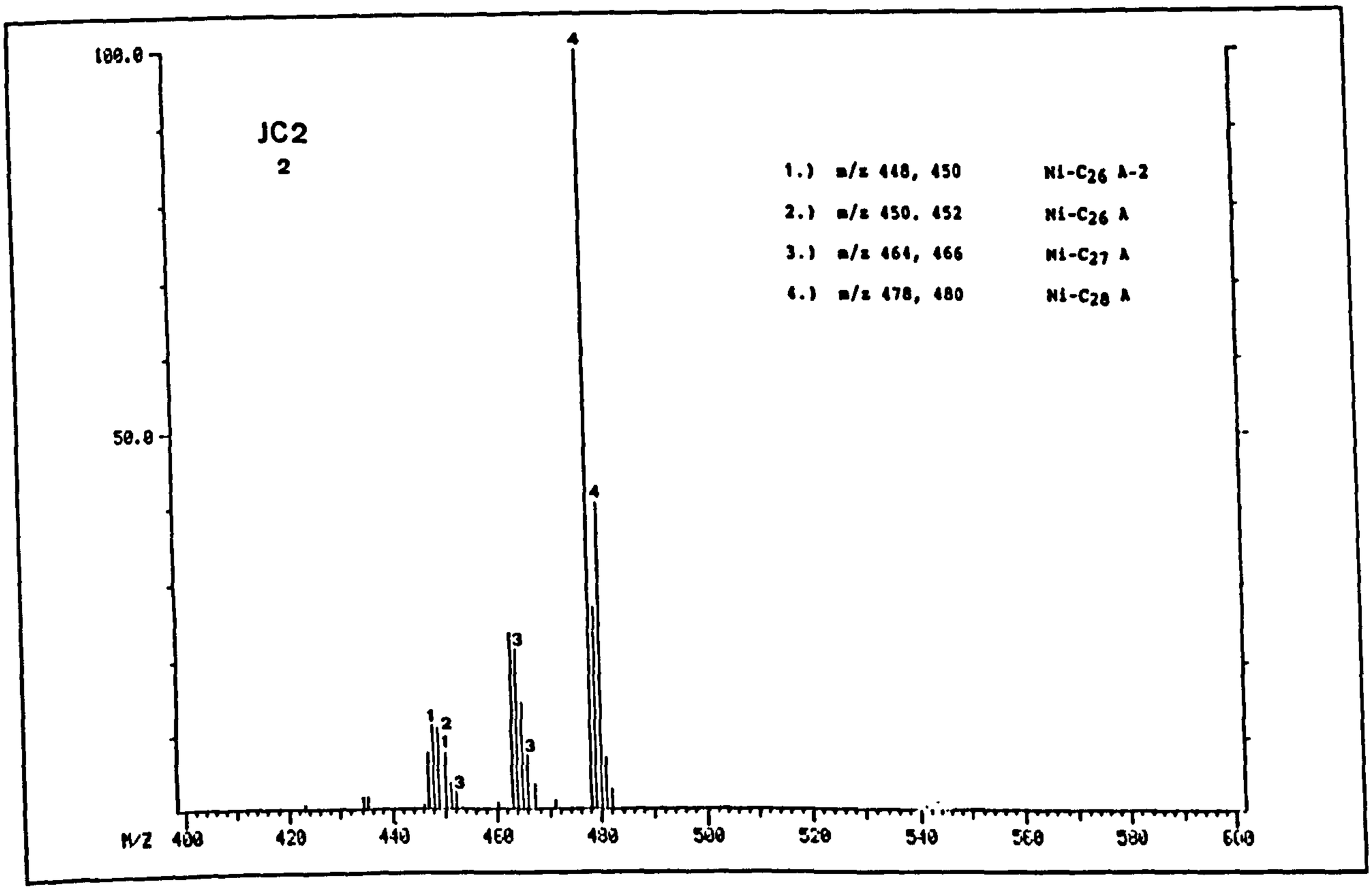


Fig.37 Summed mass spectrum of scans 731-735 (peak 2) from TIC chromatogram of JC2 shown in Fig.35.

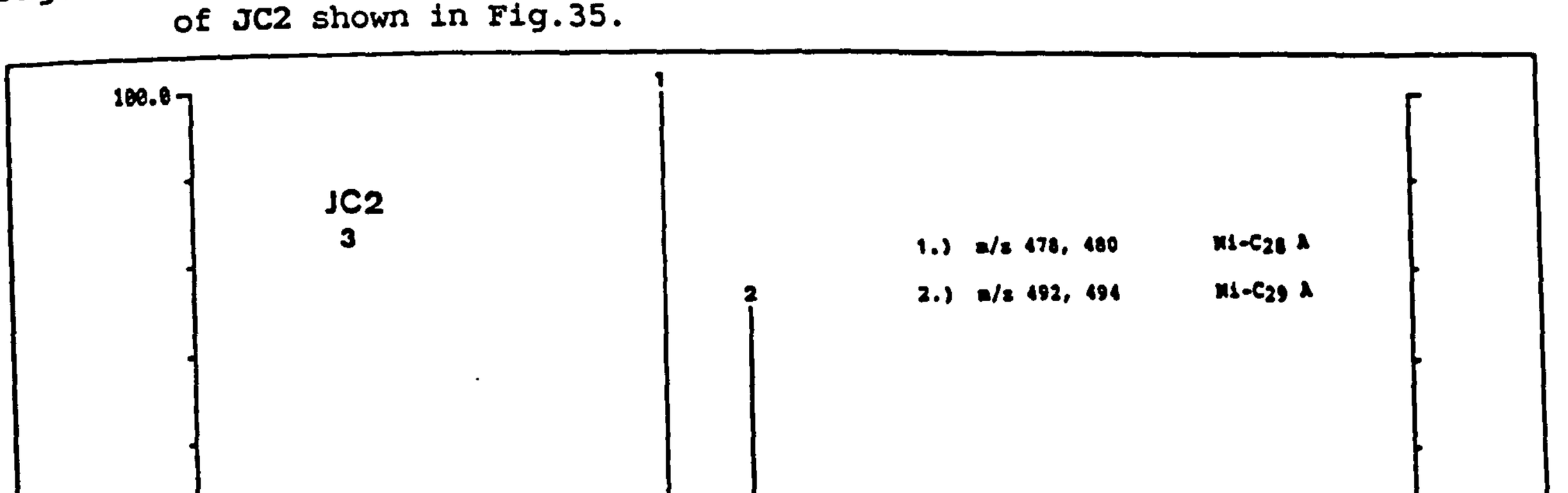


Fig.38 Summed mass spectrum of scans 749-754 (peak 3) from TIC chromatogram of JC2 shown in Fig.35.

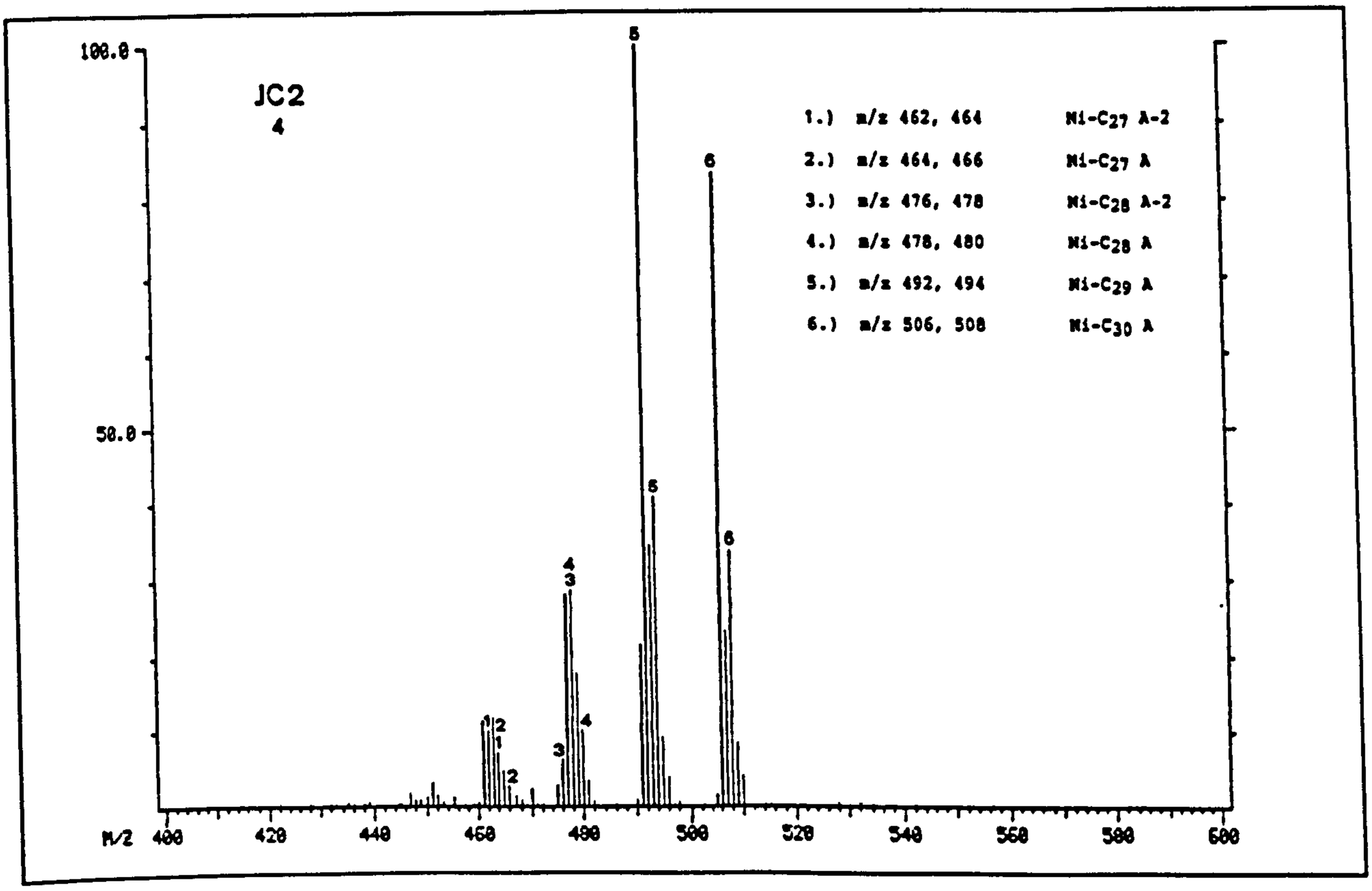


Fig.39 Summed mass spectrum of scans 778-786 (peak 4) from TIC chromatogram of JC2 shown in Fig.35.

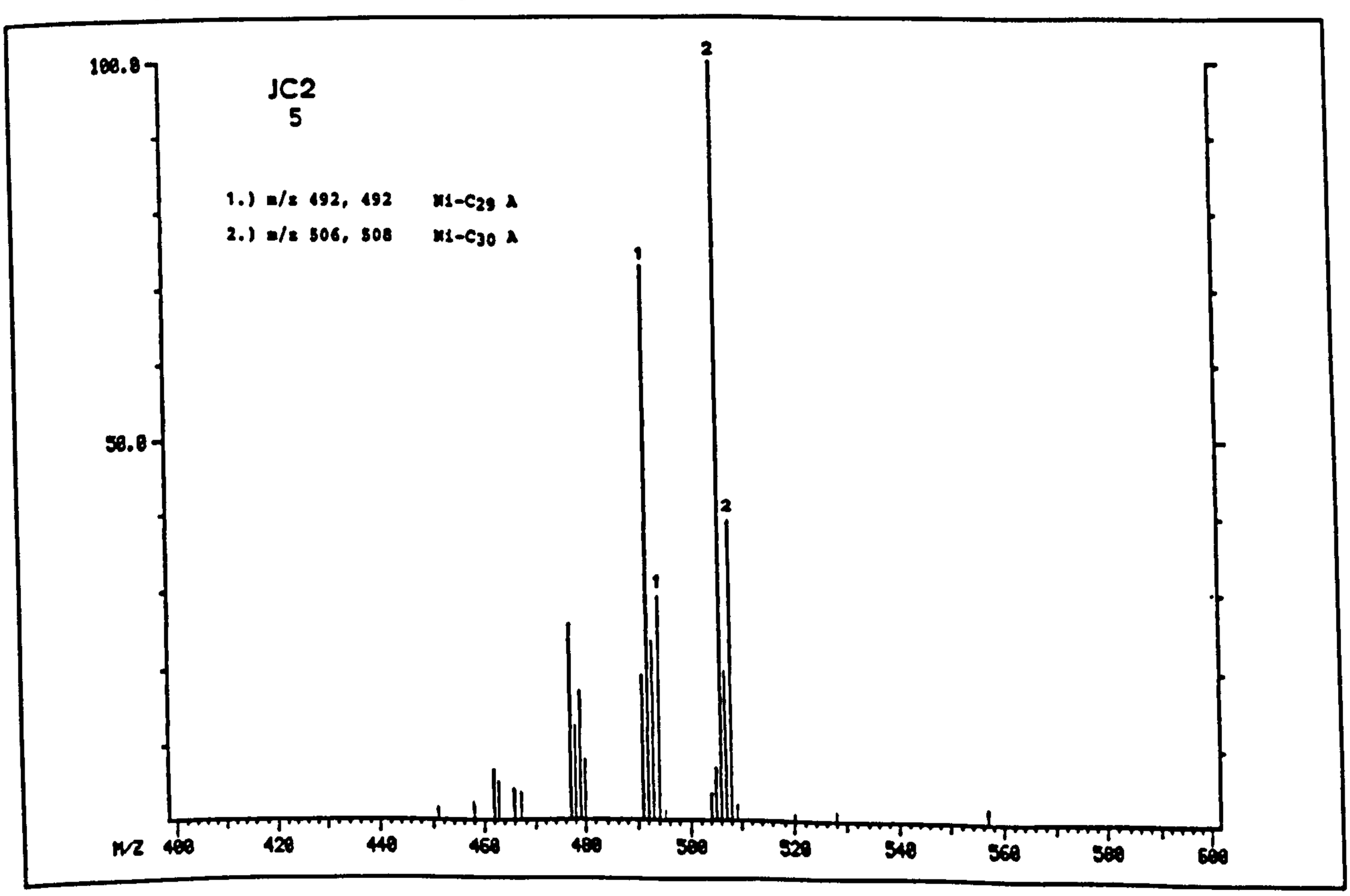


Fig.40 Summed mass spectrum of scans 797-799 (peak 5) from TIC chromatogram of JC2 shown in Fig.35.

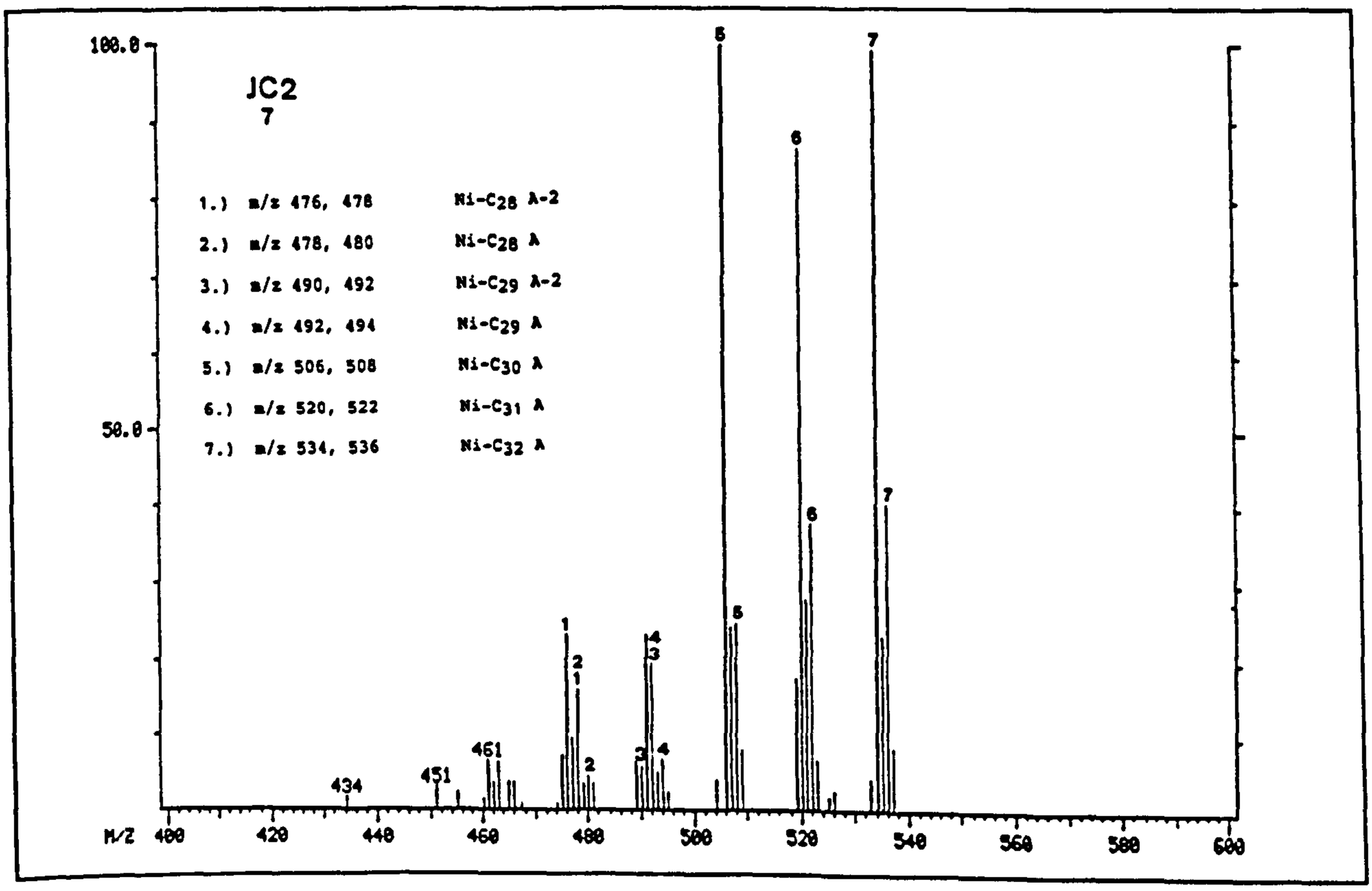


Fig.41 Summed mass spectrum of scans 826-831 (peak 7) from TIC chromatogram of JC2 shown in Fig.35.

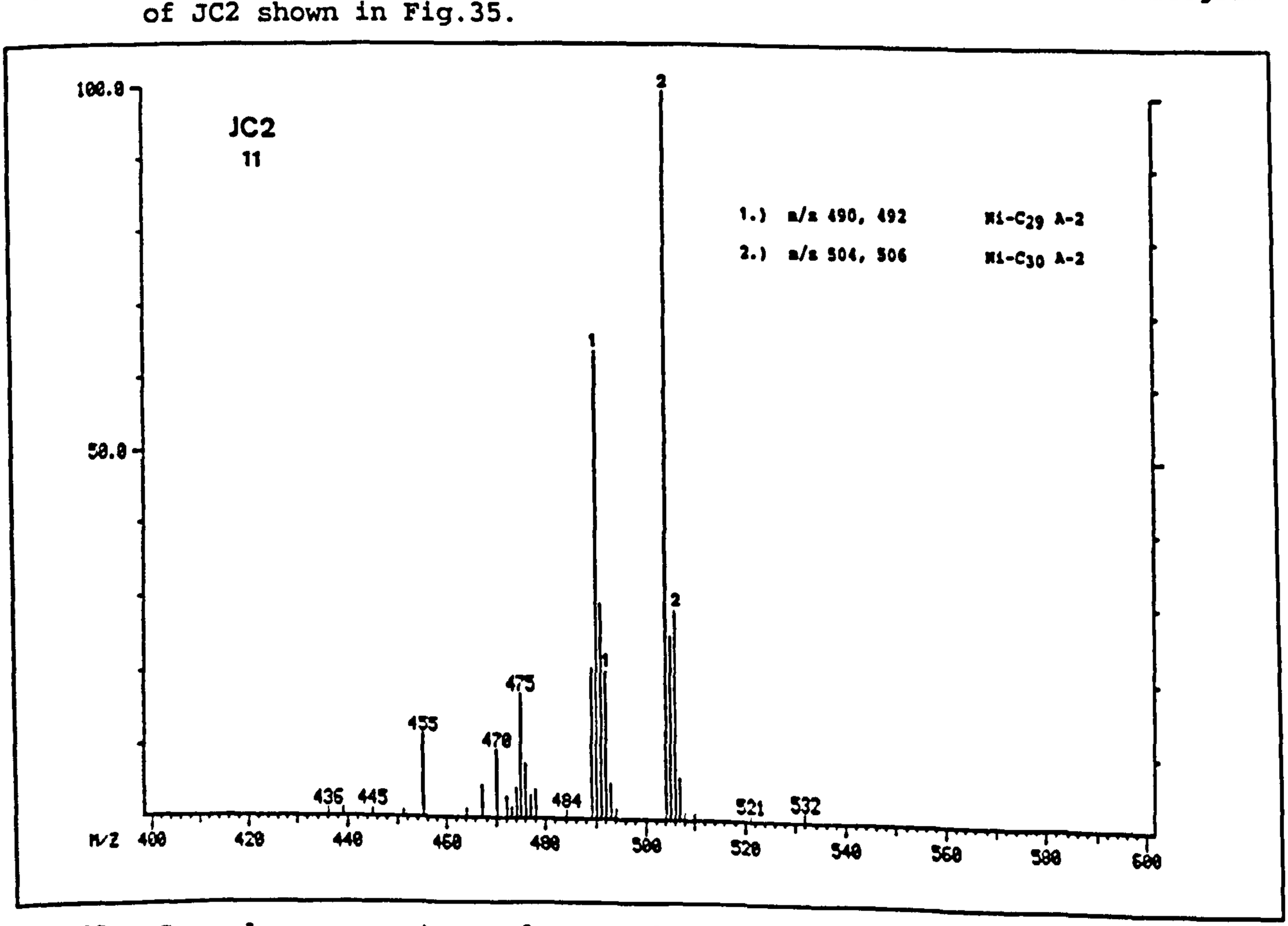


Fig.42 Summed mass spectrum of scans 874-877 (peak 11) from TIC chromatogram of JC2 shown in Fig.35.

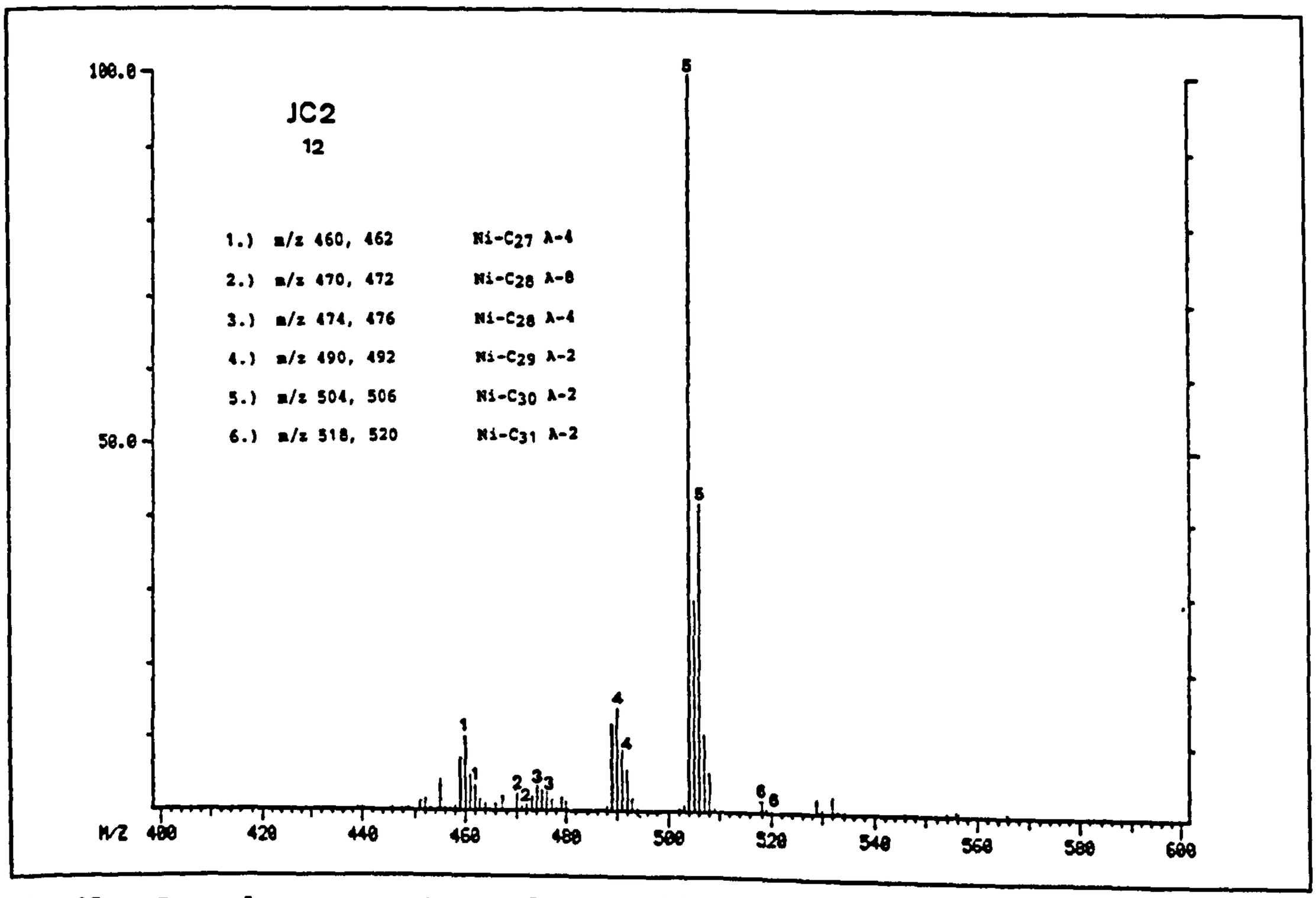


Fig.43 Summed mass spectrum of scans 905-911 (peak 12) from TIC chromatogram of JC2 shown in Fig.35.

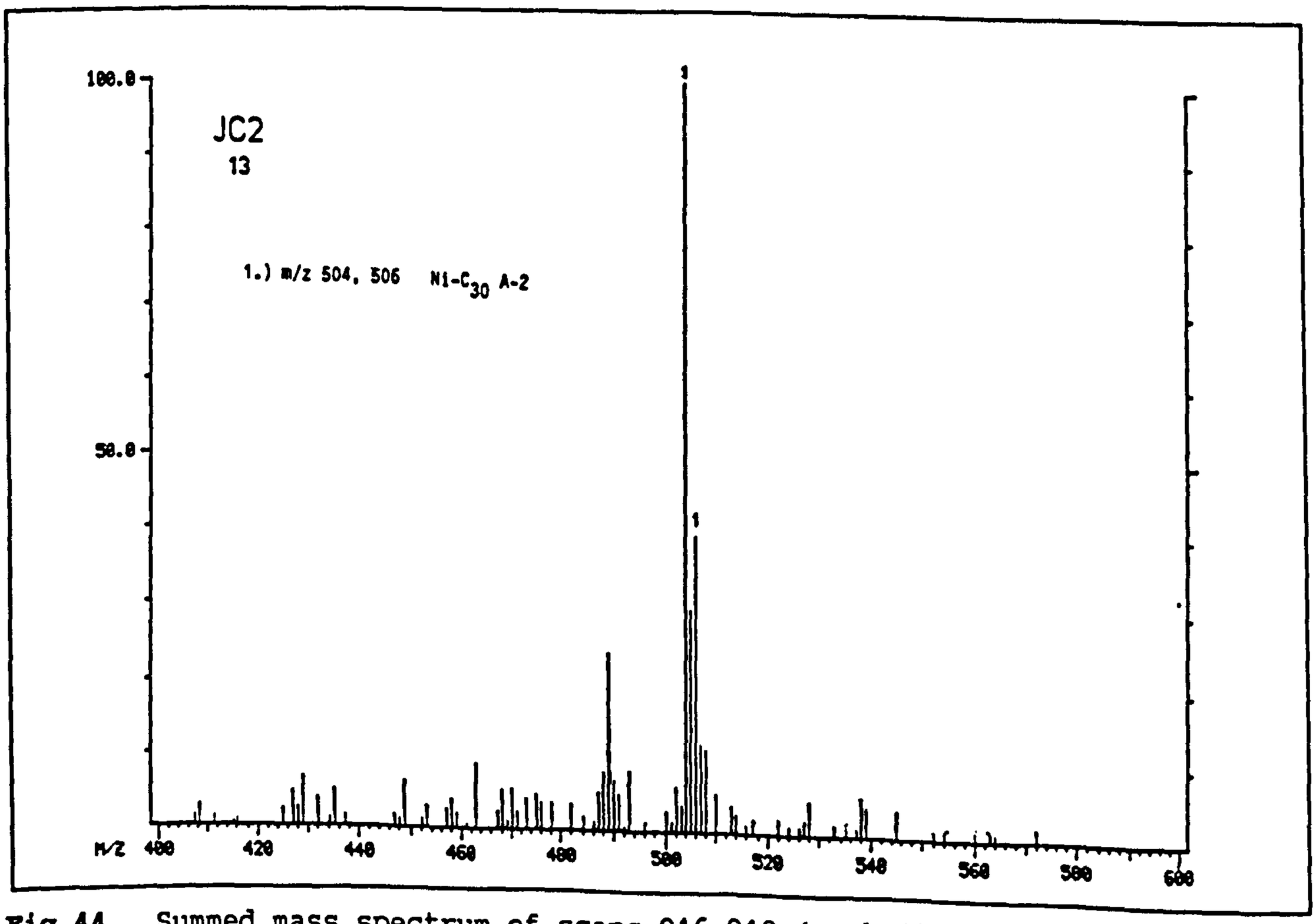


Fig.44 Summed mass spectrum of scans 916-918 (peak 13) from TIC chromatogram of JC2 shown in Fig.35.

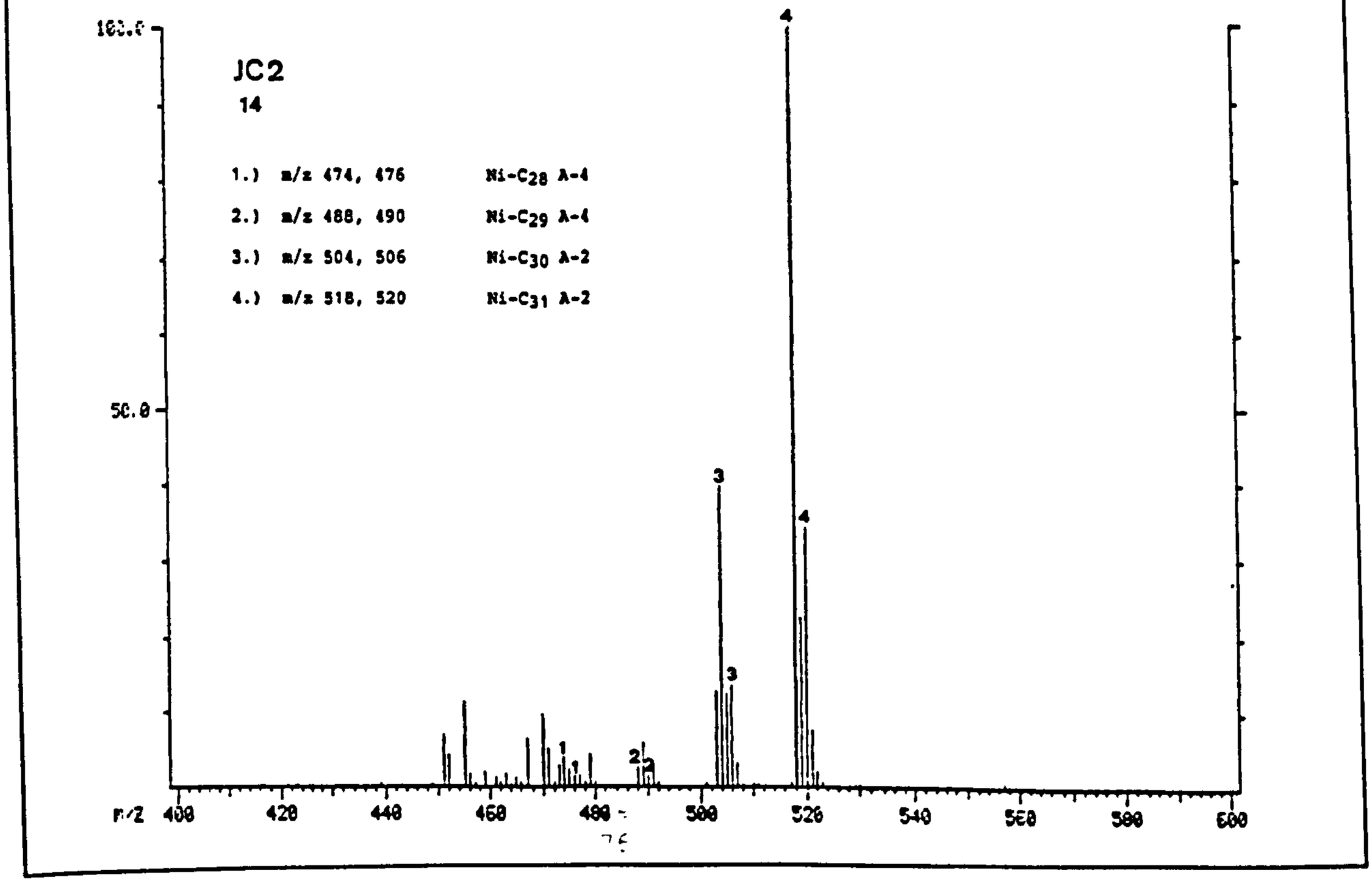


Fig.45 Summed mass spectrum of scans 925-929 (peak 14) from TIC chromatogram of JC2 shown in Fig.35.

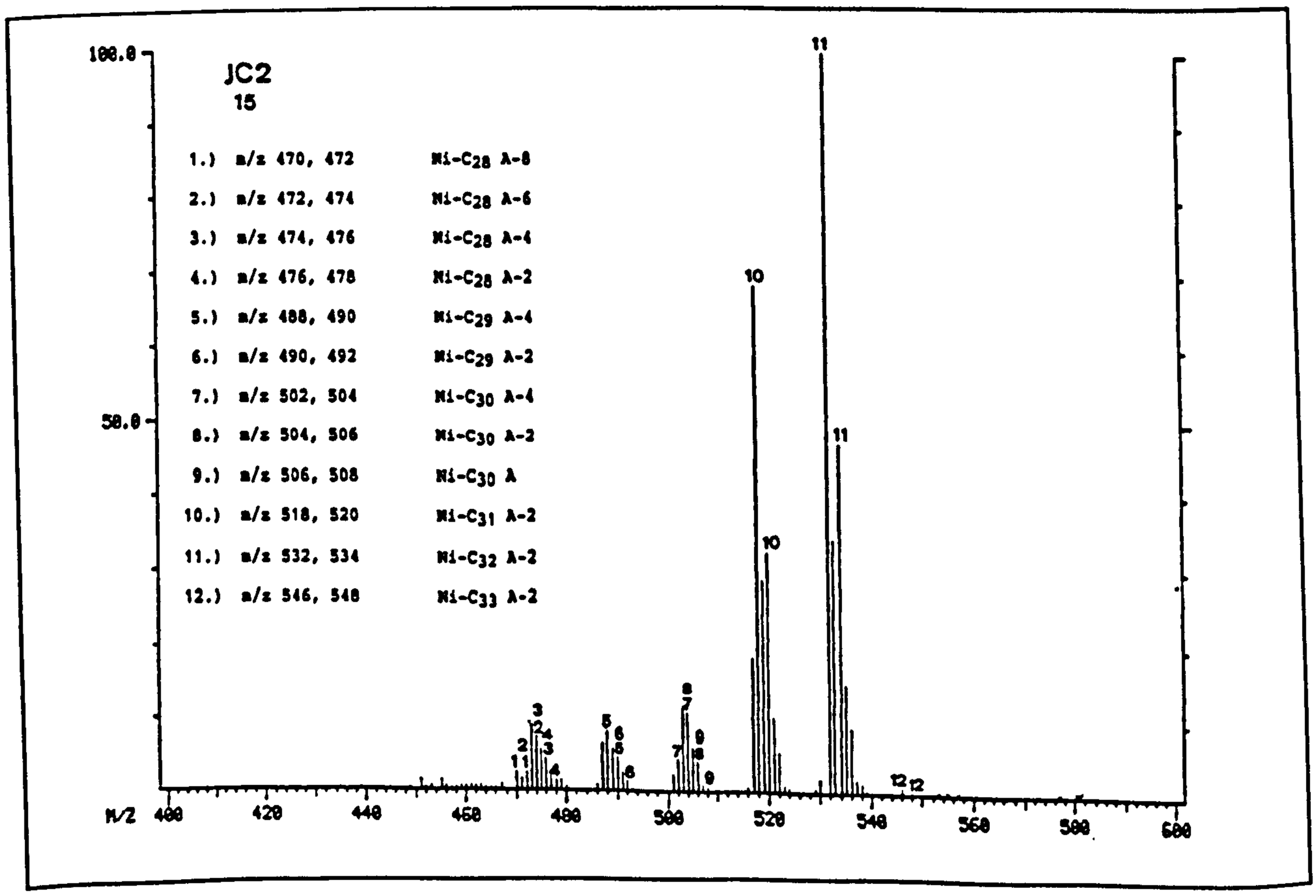


Fig.46 Summed mass spectrum of scans 954-961 (peak 15) from TIC chromatogram of JC2 shown in Fig.35.

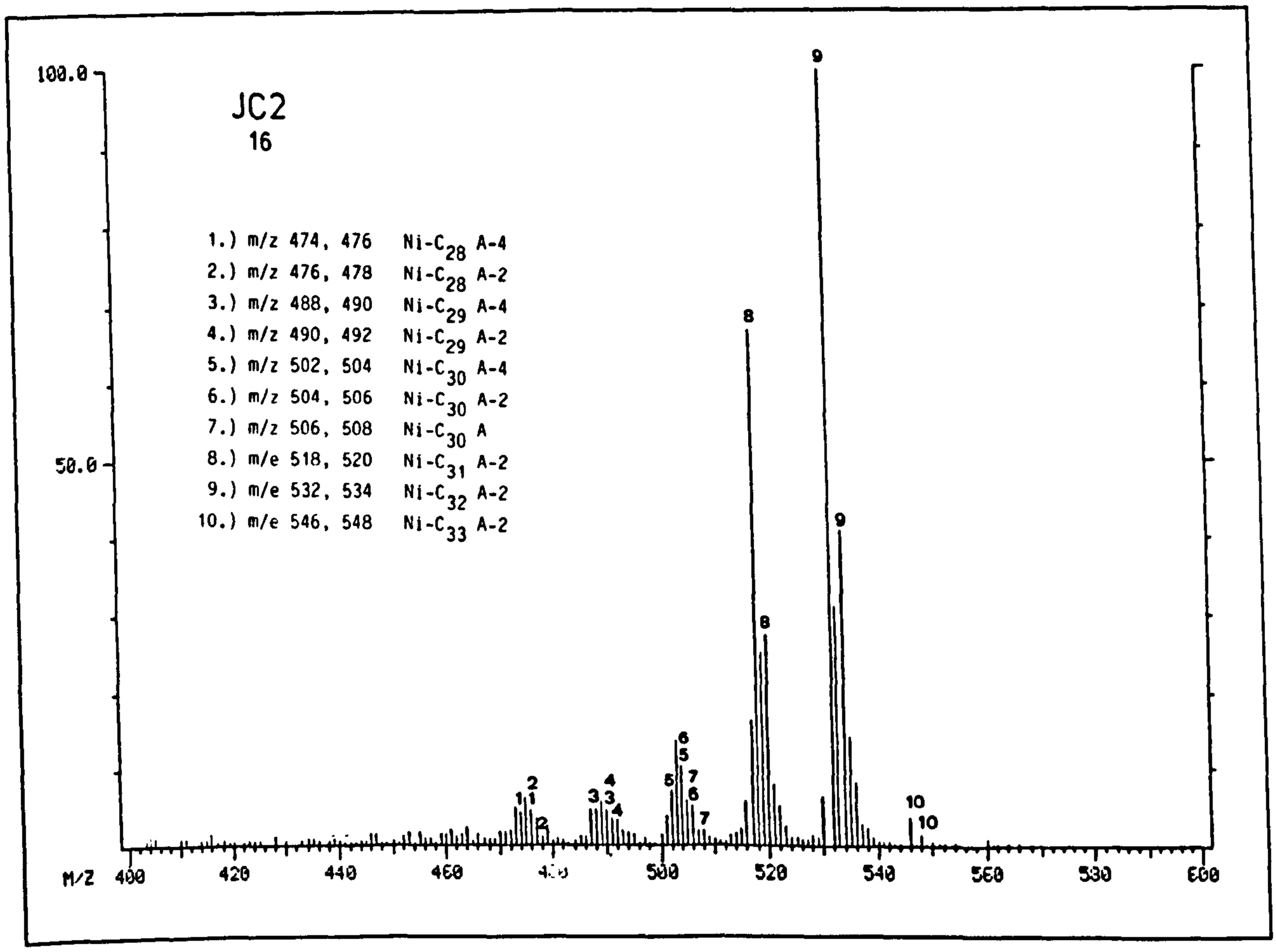


Fig.47 Summed mass spectrum of scans 963-968 (peak 16) from TIC chromatogram of JC2 shown in Fig.35.

The significant even numbered ions in the summed mass spectra shown in Figs.36-47 are accompanied by pronounced isotope peaks two masses higher. The patterns are in accordance with the isotope distribution of nickel ($^{58}Ni/^{60}Ni$) or copper ($^{63}Cu/^{65}Cu$), as presented in Fig.22. But, since copper has an odd numbered atomic weight and an even number of valences, odd numbered molecular ions are expected for copper porphyrins. Thus, we must attribute the even numbered molecular ions to nickel porphyrins. This interpretation is in agreement with the results of inductively-coupled plasma emission mass spectrometry (ICPMS) measurements of fraction JC2.

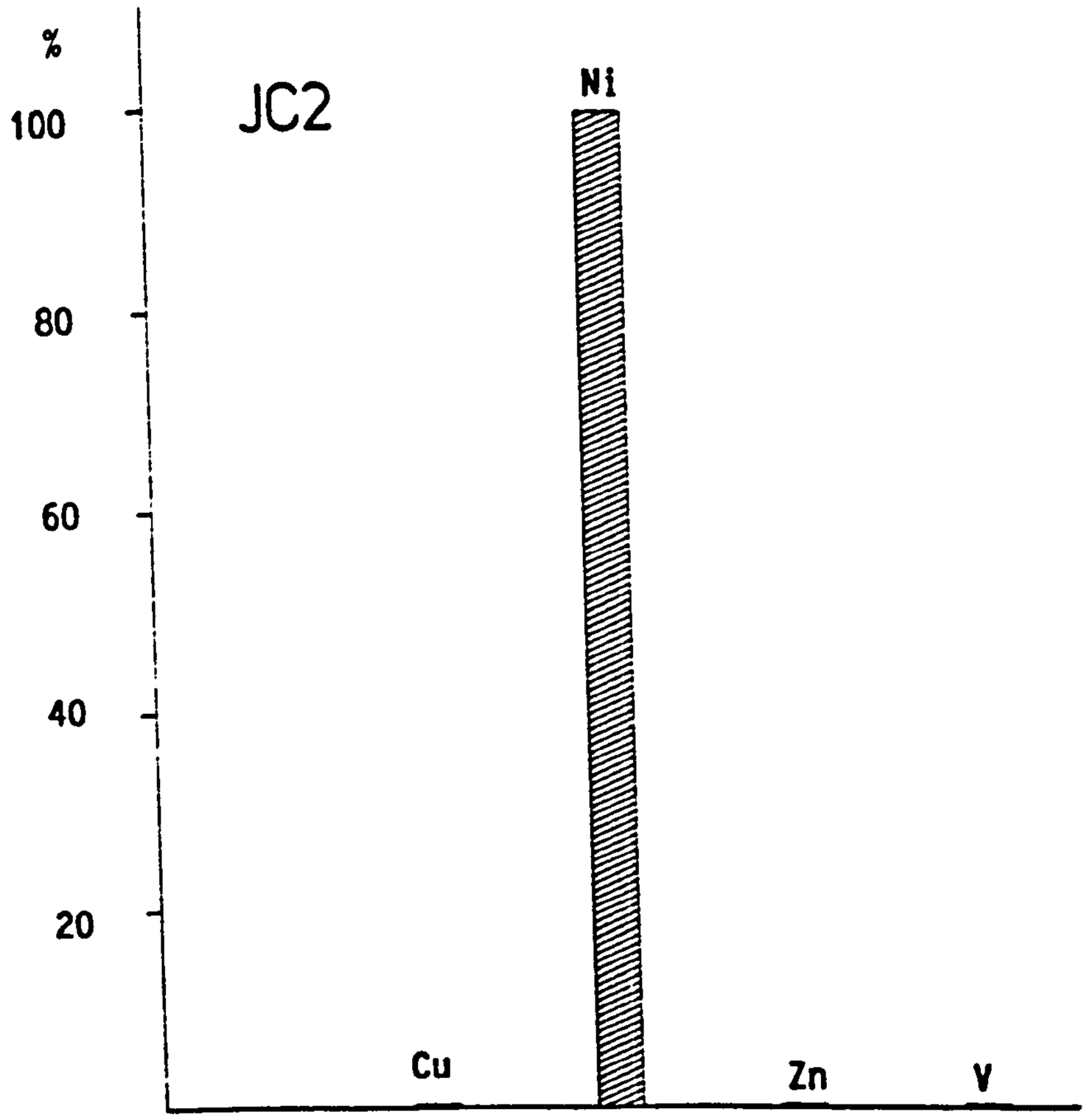


Fig.48 Graphical presentation of the metal distribution of fraction JC2 measured by ICPMS.

The ICPMS result indicates that fraction JC2 contains five transition metal elements which are known to form complexes with free base porphyrins, with nickel being the most abundant metal (99%). Owing to this simple transition metal distribution, the interpretation of the clusters of odd numbered ions, being present in all summed mass spectra and making up about 15% of the total ion current, remains doubtful. Our first assumption, deduced from ICP measurements of liquid chromatographic fractions of Marl Slate oil shale extracts (Eglinton, G., 1989), considering that some of the odd numbered (molecular?) ions could be explained as cobalt-porphyrins, does not hold true anymore. Moreover, the chromatographic behaviour of the unknowns is in agreement with the ICPMS

findings. As shown in Section I.3.2.3, various metallated octaethylporphyrins can be easily base line separated by GC. Accordingly, for hypothetical cobalt porphyrins in fraction JC2 a separation from nickelporphyrins is expected, provided that both metals form chelates with the same free base porphyrin. But in none of the cases examined by mass selected detection could a chromatographic resolution be obtained. In order to demonstrate the point, a respective pair of ions at m/z 506, interpreted as $^{58}Ni\text{-}C_{30}$ A/$^{60}Ni\text{-}C_{30}$ A-2 , and at m/z 507, previously interpreted as Co-C_{30} A, is shown in the figure below.

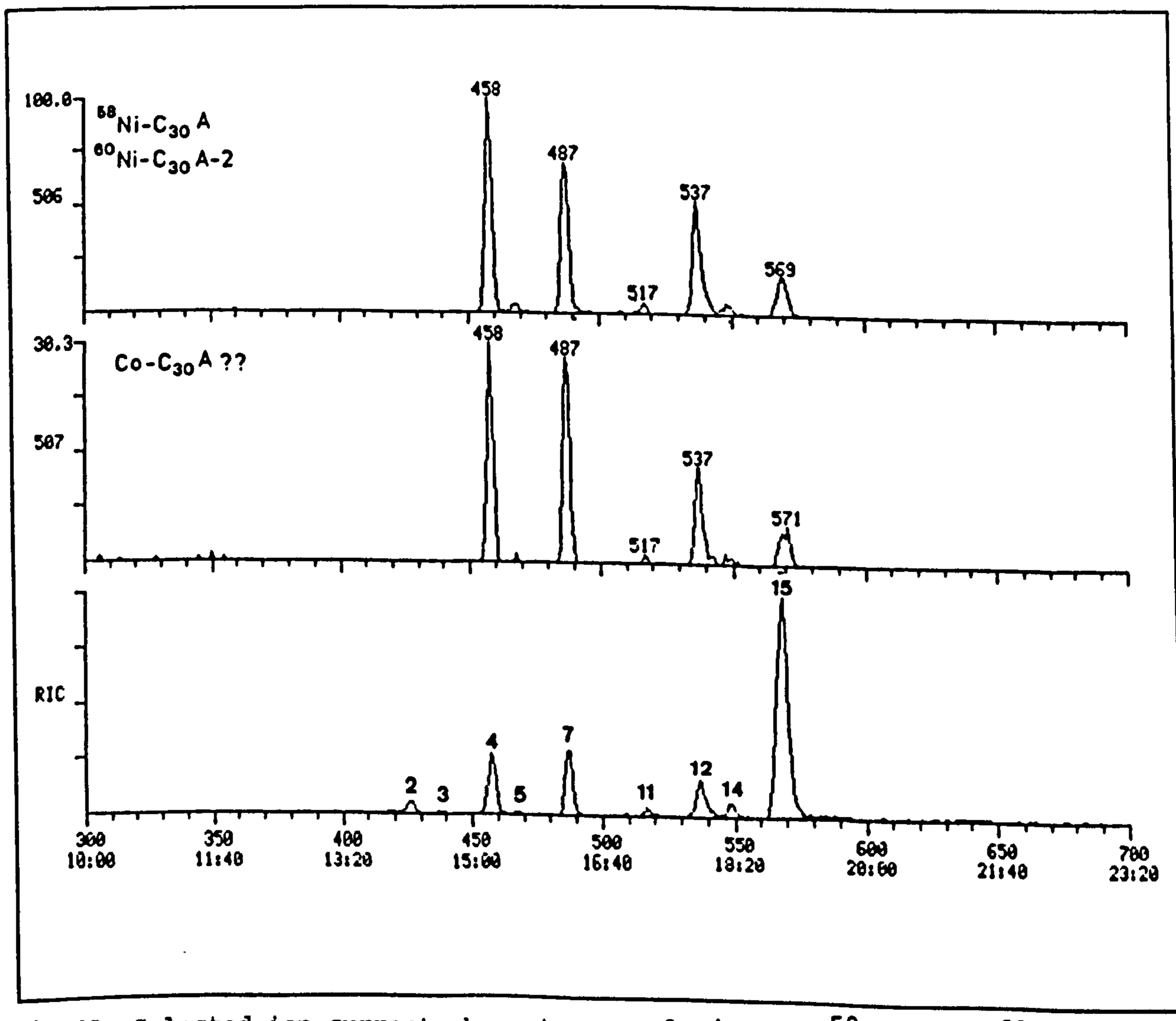


Fig.49 Selected ion current chromatogram of m/z 506 ($^{58}Ni\text{-}C_{30}$ A/^{60}-Ni-C_{30} A-2) and m/z 507 (hypothetical Co-C_{30} A). Both ions appear at the same retention temperature and hence m/z 506 can not be Co-C_{30} A.

Thus, the questions whether the undefined ions are molecular- or fragment ions, or of porphyrinogenic or of non-porphyrinogenic nature could not be answered from a separation on a PS-090 coated column by means of EI-MS detection, although their mass spectrometrical behaviour would correspond to tetrapyrrole systems.
Deduced from the summed EI mass spectra, in the following Table 3 the individual nickel alkyl porphyrins are listed and correlated to single GC-peaks.
Quantification was carried out on the abs. ion intensities of the single molecular ions. A possible enhancement of the ion current by ^{13}C- and /or ^{60}Ni-isotope peaks has been taken into account. The respective results are given in % of the total ion current, the most abundant component of a GC-peak is bold typed.

Table 3

GC peak No.	Molecular Ion Distribution	Semi-Systematic Characterisation
1	1. m/z 464, 468 2. m/z 478, 480	Ni-C_{27} A (0.2 %) Ni-C_{28} A (0.1 %)
2	1. m/z 448, 450 2. m/z 450, 452 3. m/z 464, 466 4. m/z 478, 480	Ni-C_{26} A-2 (0.1 %) Ni-C_{26} A (0.1 %) Ni-C_{27} A (0.50 %) Ni-C_{28} A (1.5 %)
3	1. m/z 478, 480 2. m/z 492, 494	Ni-C_{28} A (0.20 %) Ni-C_{29} A (0.20 %)
4	1. m/z 462, 464 2. m/z 464, 466 3. m/z 476, 478 4. m/z 478, 480 5. m/z 492, 494 6. m/z 506, 508	Ni-C_{27} A-2 (0.28 %) Ni-C_{27} A (0.20 %) Ni-C_{28} A-2 (0.30 %) Ni-C_{28} A (1.1 %) Ni-C_{29} A (3.70 %) Ni-C_{30} A (3.50 %)
5	1. m/z 492, 494 2. m/z 506, 508	Ni-C_{29} A (0.10 %) Ni-C_{30} A (0.14 %)
7	1 m/z 476, 478 2 m/z 478, 480 3 m/z 490, 492 4 m/z 492, 494 5 m/z 506, 508 6 m/z 520, 522 7 m/z 534, 536	Ni-C_{28} A-2 (0.5 %) Ni-C_{28} A (0.11 %) Ni-C_{29} A-2 (0.1 %) Ni-C_{29} A (0.50 %) Ni-C_{30} A (2.8 %) Ni-C_{31} A (3.5 %) Ni-C_{32} A (3.5 %)

GC peak No.	Molecular Ion Distribution	Semi-Systematic Characterisation
11	1. m/z 490, 492 2. m/z 504, 506	Ni-C_{29} A-2 (0.45 %) Ni-C_{30} A-2 (0.60 %)
12	1. m/z 460, 462 2. m/z 470, 472 3. m/z 474, 476 4. m/z 490, 492 5. m/z 504, 506 6. m/z 518, 520	Ni-C_{27} A-4 (0.4 %) Ni-C_{28} A-8 (0.1 %) Ni-C_{28} A-4 (0.14 %) Ni-C_{29} A-2 (0.60 %) Ni-C_{30} A-2 (5.0 %) Ni-C_{31} A-2 (0.05 %)
13	1. m/z 504, 506	Ni-C_{30} A-2 (0.5 %)
14	1. m/z 474, 476 2. m/z 488, 490 3. m/z 504, 506 4. m/z 518, 520	Ni-C_{28} A-4 (0.09 %) Ni-C_{29} A-4 (0.07 %) Ni-C_{30} A-2 (0.30 %) Ni-C_{31} A-2 (1.5 %)
15	1. m/z 470, 472 2. m/z 472, 474 3. m/z 474, 476 4. m/z 476, 478 5. m/z 488, 490 6. m/z 490, 492 7. m/z 502, 504 8. m/z 504, 506 9. m/z 506, 508 10. m/z 518, 520 11. m/z 532, 534 12. m/z 546, 548	Ni-C_{28} A-8 (0.3 %) Ni-C_{28} A-6 (0.7 %) Ni-C_{28} A-4 (1.3 %) Ni-C_{28} A-2 (0.6 %) Ni-C_{29} A-4 (1.6 %) Ni-C_{29} A-2 (0.75 %) Ni-C_{30} A-4 (0.7 %) Ni-C_{30} A-2 (1.4 %) Ni-C_{30} A (0.5 %) Ni-C_{31} A-2 (16.6 %) Ni-C_{32} A-2 (24.3 %) Ni-C_{33} A-2 (0.2 %)
16	1. m/z 474, 476 2. m/z 476, 478 3. m/z 488, 490 4. m/z 490, 492 5. m/z 502, 504 6. m/z 504, 506 7. m/z 518, 520 9. m/z 532, 534 10. m/z 546, 548	Ni-C_{28} A-4 (0.2 %) Ni-C_{28} A-2 (0.29 %) Ni-C_{29} A-4 (0.2 %) Ni-C_{29} A-2 (0.15 %) Ni-C_{30} A-4 (0.3 %) Ni-C_{30} A-2 (0.6 %) Ni-C_{31} A-2 (6.9 %) Ni-C_{32} A-2 (8.1 %) Ni-C_{33} A-2 (0.1 %)

Table 3: Nickelporphyrin distribution of fraction JC2 (Fig.35), separated on a 20m x 0.3 mm, PS-090 coated glass capillary column.

The example of fraction JC2 demonstrates that a flood of information can be derived from a simple geoporphyrin total ion current record, exhibiting a total of only twelve separated peaks, by molecular ion specific search. In order to organise, to compress and to represent the information of the tables more distinctly, the data of Table 2 and the following GC/MS records were transmitted to graphic presentations as shown in Fig.50-51.

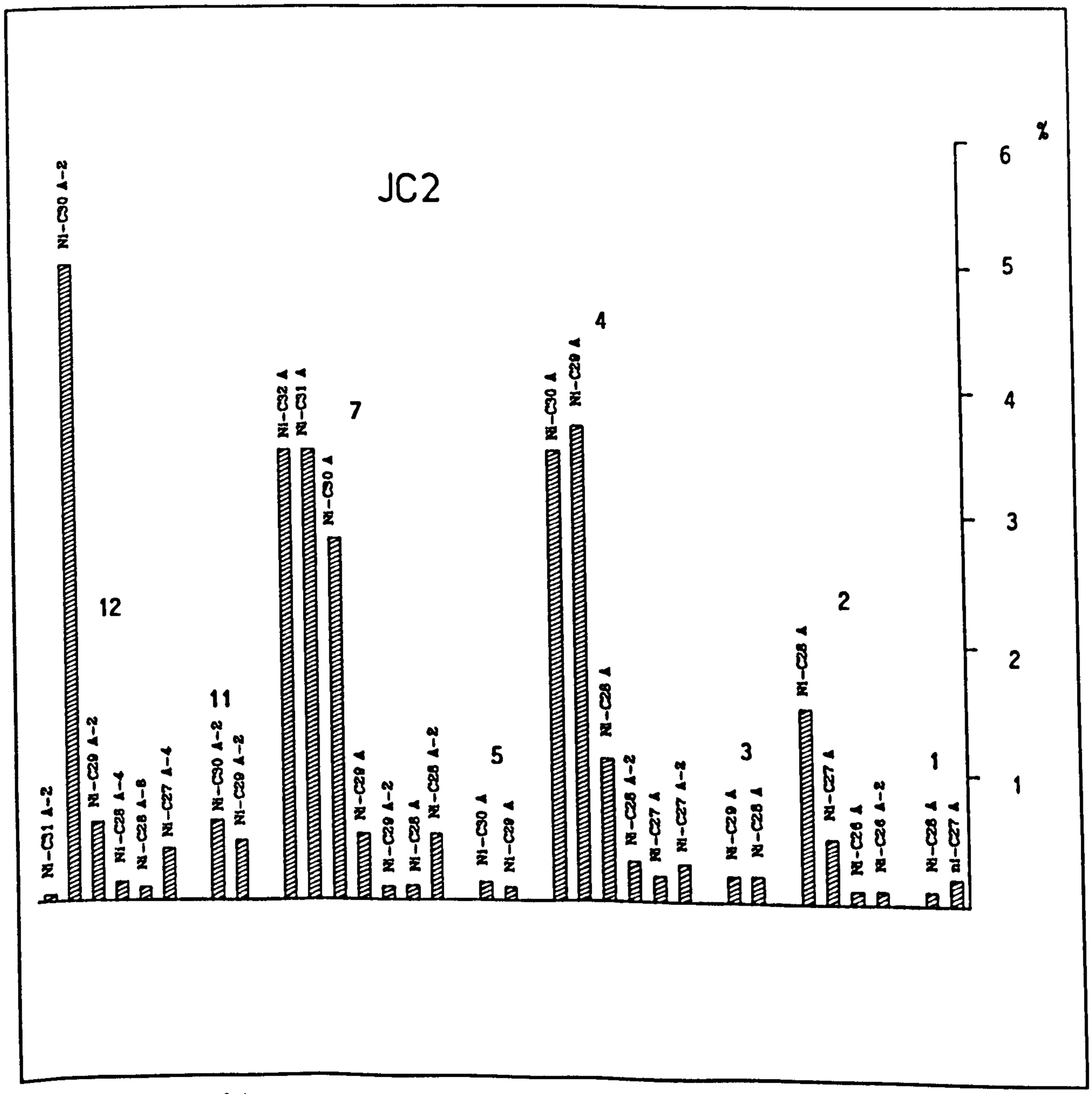


Fig.50 Graphic presentation of the nickel porphyrin distribution of GC-peaks 1, 2, 3, 4, 5, 7, 11 and 12 of fraction JC2 (see Fig.35).

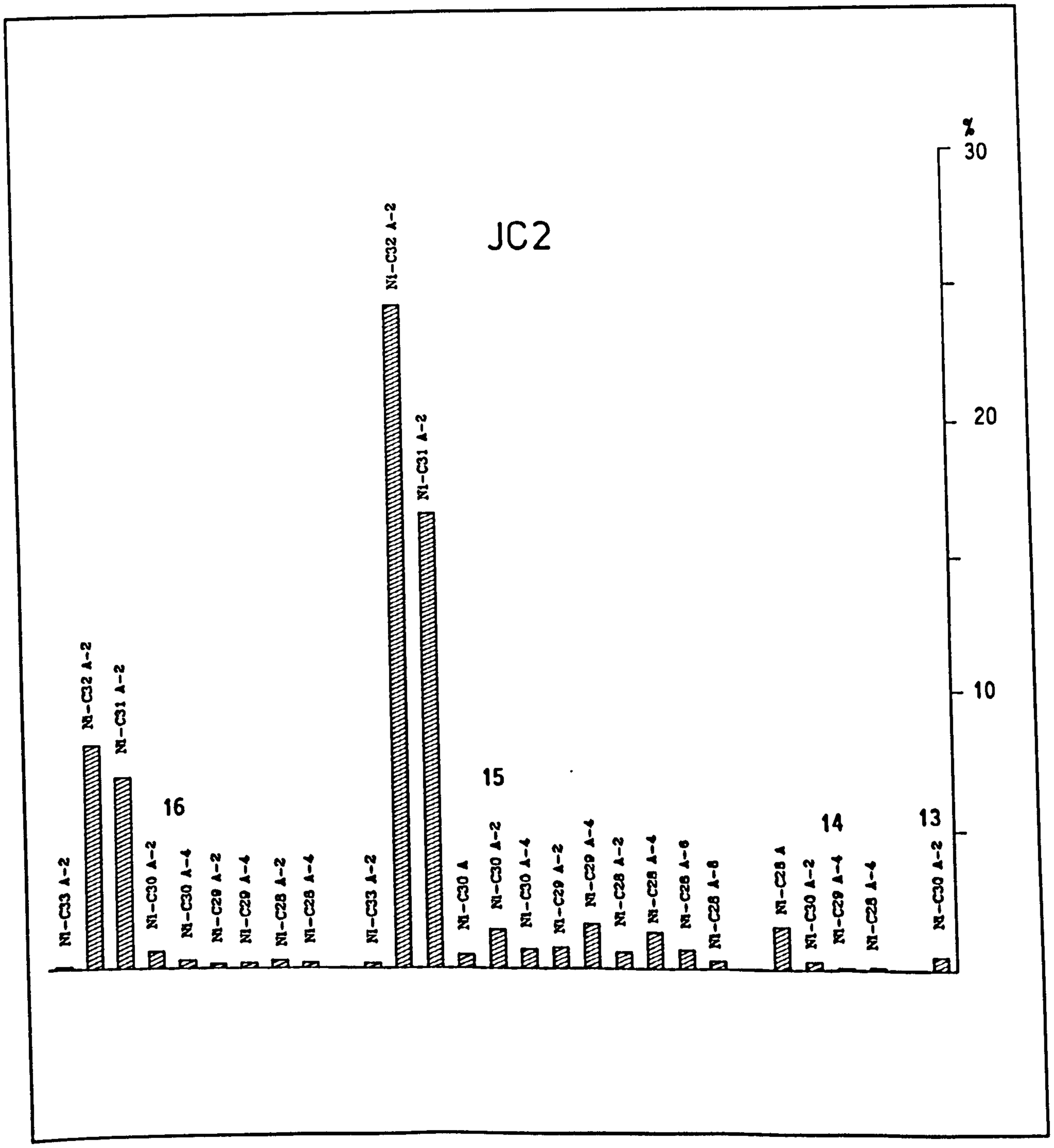


Fig.51 Graphic presentation of the nickel porphyrin distribution of GC-peaks 13, 14, 15 and 16 of fraction JC2 (see Fig.35).

V.2.1.3.1.2. GC/MS Analysis of JC2 on an OV-61-OH (33% Phenyl) Coated Column

In Section I.3.2.2. it was stressed that the separation of porphyrin isomers can be improved by more polar coatings. The two chromatograms of fractions JC2 below, carried out on a PS-090 (20% phenyl) and on a OV-61-OH (33% phenyl) coated capillary column, respectively, showed that JC2 is better resolved on a more polar stationary phase, in fact.

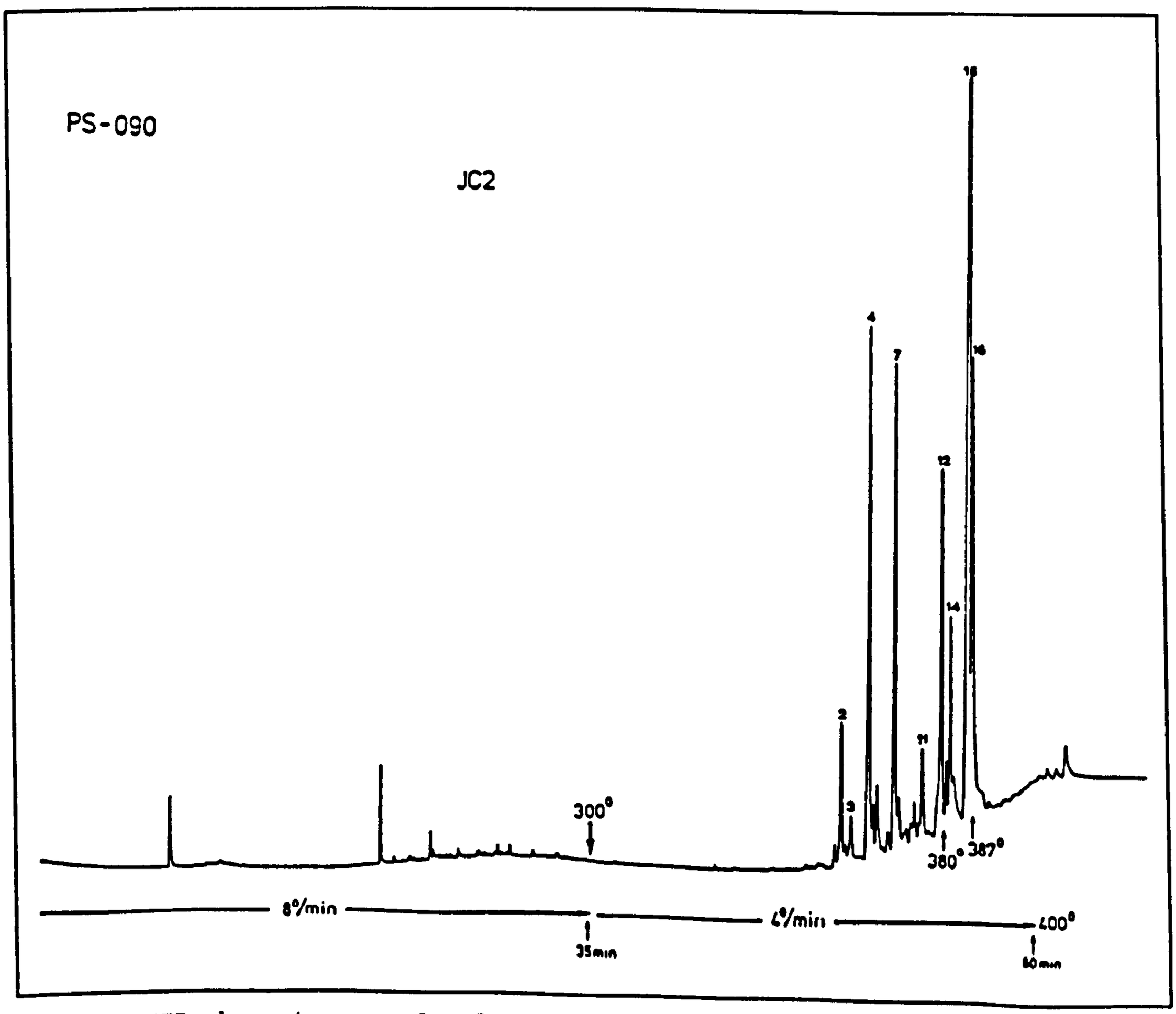


Fig.52a FID chromatogram of JC2 on a PS-090 coated column.

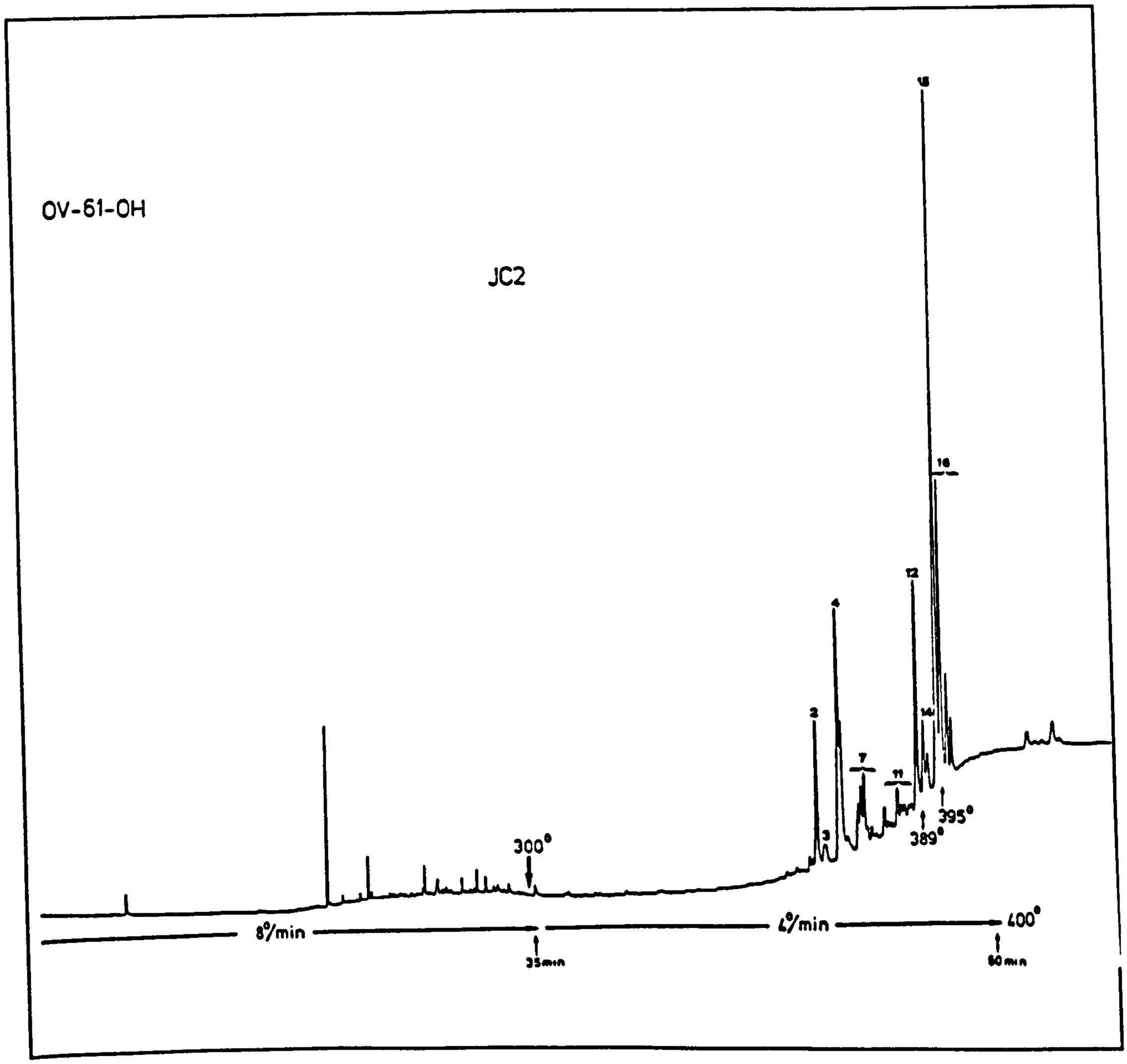


Fig.52b FID chromatogram of JC2 on a OV-61-OH coated column

As expected the cluster of nickel-porphyrins shifts to about 10°C higher retention temperatures. An unambiguous assignment of the single peaks of these two records is therefore only possible by subsequent GC/MS examination.

A comparison of the FID record and the TIC chromatogram on the OV-61-OH coated column exhibits also reduced resolution and some trace components have disappeared on their way from the column exit to the ion source, although the same column has been used. Nevertheless, the most intense GC peaks are split into multiplets and the resulting mass spectra are simpler and thus more informative.

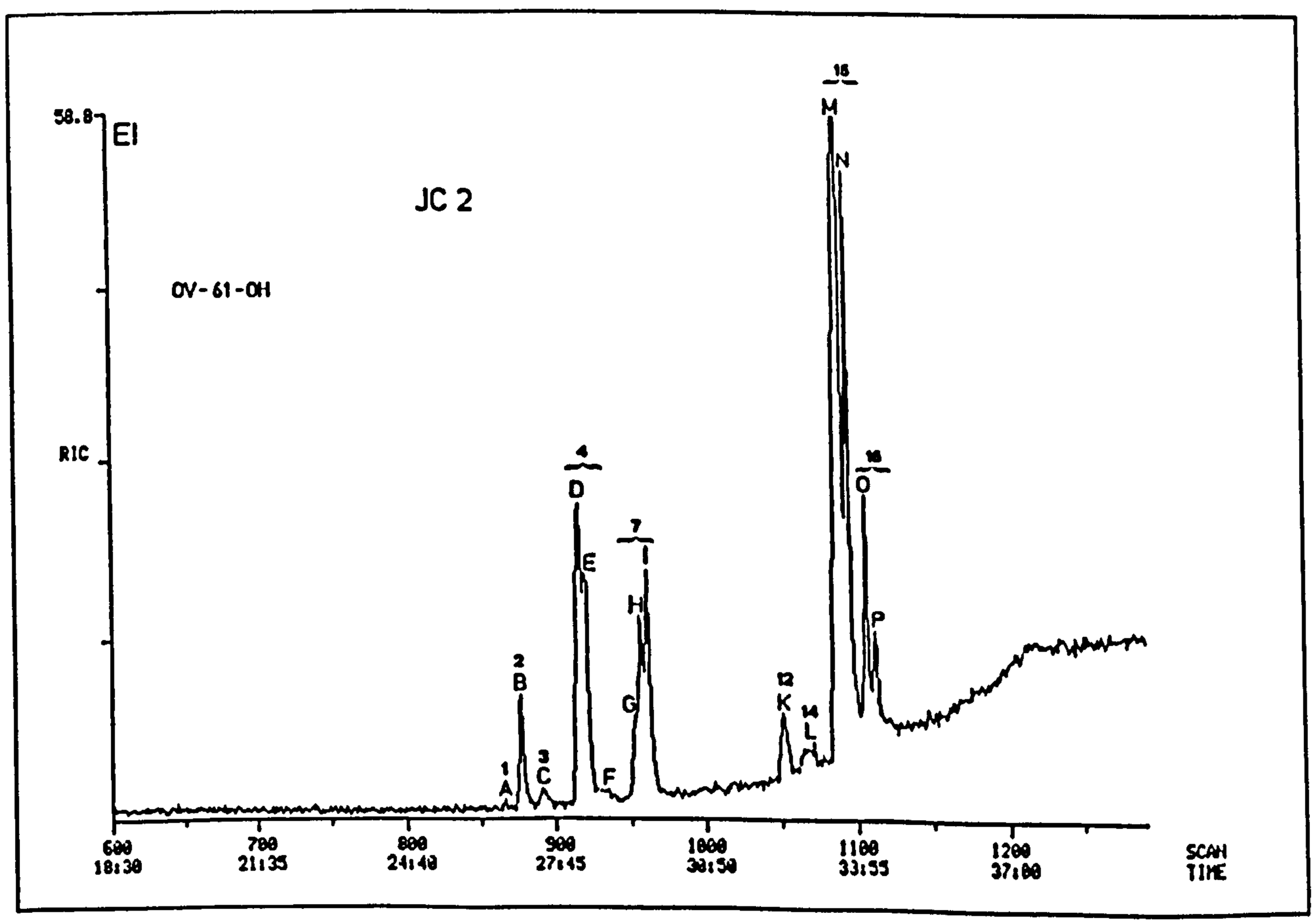


Fig.53 TIC chromatogram of JC2 on an OV-61-OH coated capillary column. The single peaks are termed with capital letters. The numbers respect to the former assignment as shown in Figs.52a-b.

Representative summed mass spectra are shown in Figs.54-56. From these spectra which are simplier than those obtained with the less polar PS-090 coated capillary the nature of the unknown odd numbered ions discussed in Section V.2.1.3.1.1 can be explained.

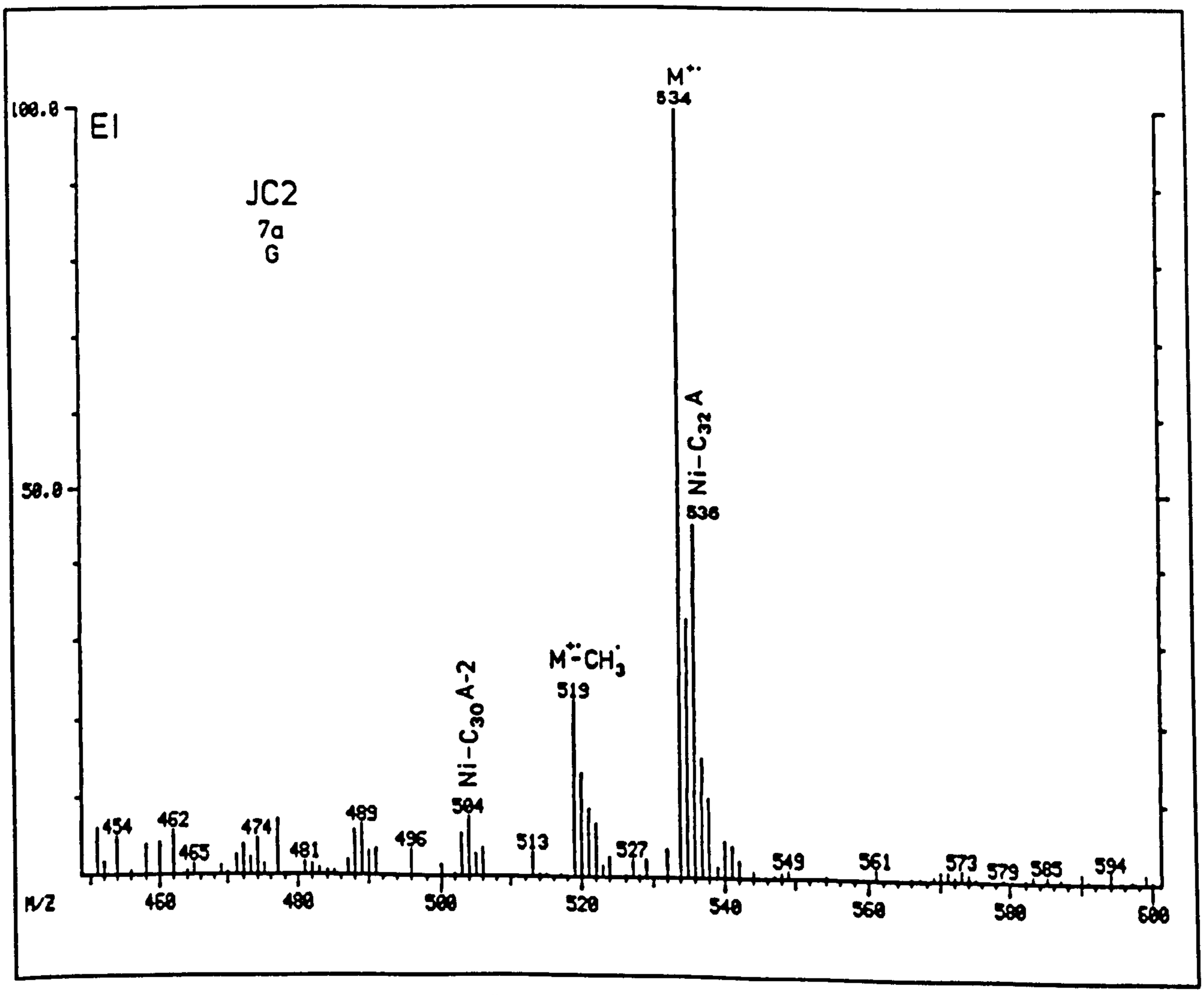


Fig.54 Summed mass spectrum of scans 494-501 (peak 7a, G) from TIC chromatogram of JC2 shown in Fig.53

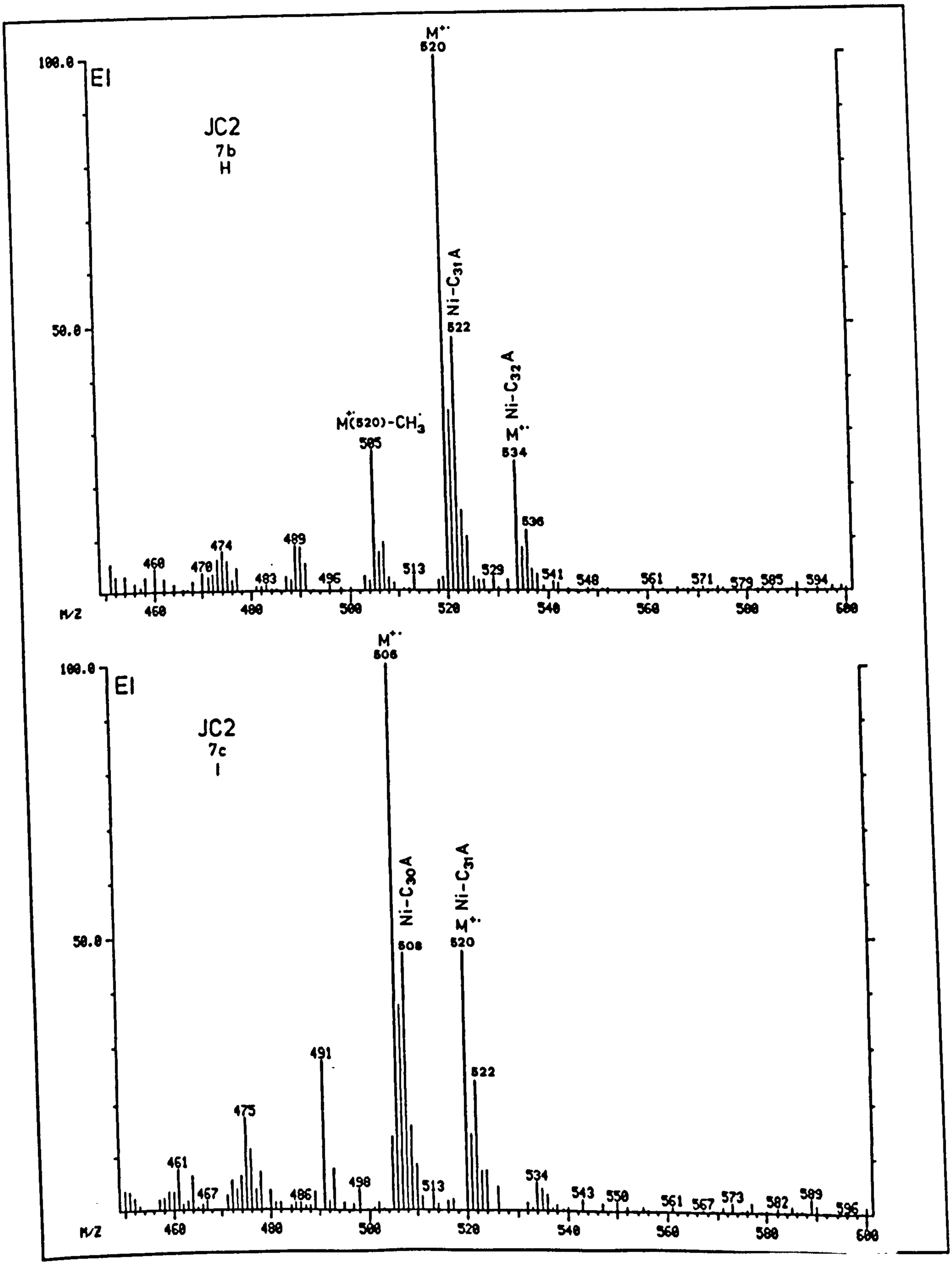


Fig.55 Summed mass spectra of scans 502-508 (peak 7b, H) and of scans 509-517 (peak 7c, I) from TIC chromatogram of JC2 shown in Fig.53.

From the cleaner EI-spectra it is evident, that the isotope pattern of most of the odd numbered ions fits to nickel. Moreover, these ions are accompanied without exception by even numbered molecular ions 15 atomic mass units (a.m.u.) higher, which were already interpreted as nickel porphyrins. Therefore, it can be supposed that most of the odd numbered ions are fragments resulting from α-cleavage of alkyl substituents($M^{+\bullet} - CH_3\bullet$) rather than molecular ions.

This fragmentation has been already reported with by Baker et al.(Baker, 1966; Baker, E.W. et al.,1967) who tried to suppress it in direct probe petroporphyrin analysis by low energy ionisation (12 eV). However, the extent of fragment ion formation was unexpected. The EI-spectra of various metalloporphyrin standards, introduced for the first time through a capillary column, showed only little fragmentation of peripheral substituents (<10%, see also Clezy, P.S. et al., 1974) in comparison to values obtained from direct probe introduction (Gill,1984e). The fragmentation behaviour of metalloporphyrins with four equivalently substituted pyrrole rings, as exemplified by 2,3,7,8,12,13,17,18-octaethylporphyrin, is obviously not representative for the extent of fragmenta- tion of metalloporphyrins in general. (In other words, we chose the wrong free base in order to synthesise metalloporphyrin stan- dards, shown in Fig.21a-f).

If the porphyrin bears additional exocyclic rings, which is common for many petroporphyrins, the fragmentation of alkyl substituents seems to be more pronounced and the assignment of unknown metallopetroporphyrins, particularly with complex isotope patterns can be difficult (e.g. if in column liquid chromatographic fractionation cobalt- and nickel porphyrins co-elute).

In such case CI/NH_3 is recommended, supplementary to (more sensitive) electron impact ionisation, as pointed out in the following section.

The distribution of nickelporphyrins found in the TIC chromatogram of Fig. 53 is presented in Figs.57 and 59.

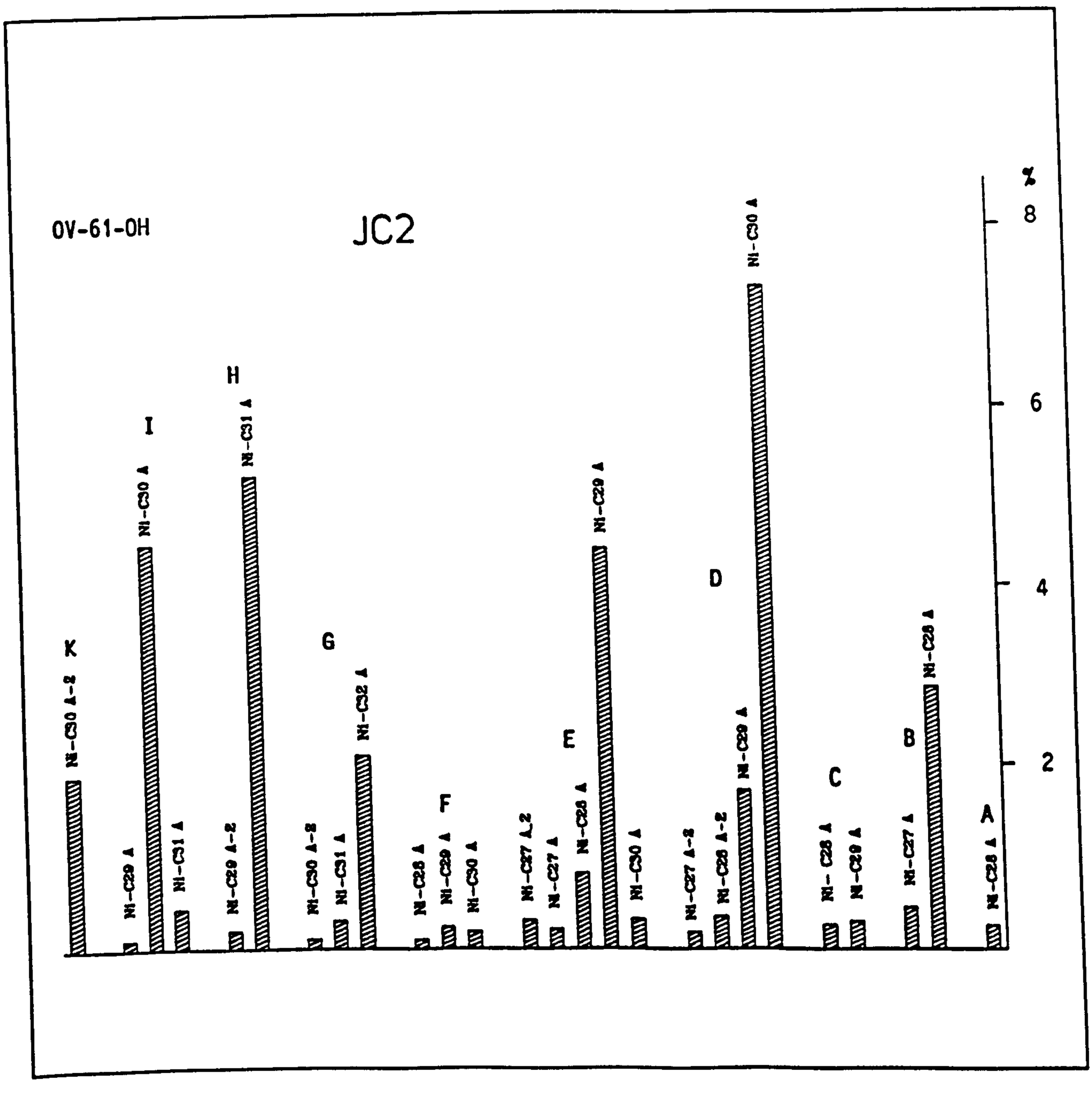


Fig.57 Graphic presentation of the nickel porphyrin distribution of GC-peaks A-K of JC2, analysed on an OV-61-OH coated column. See Fig.53.

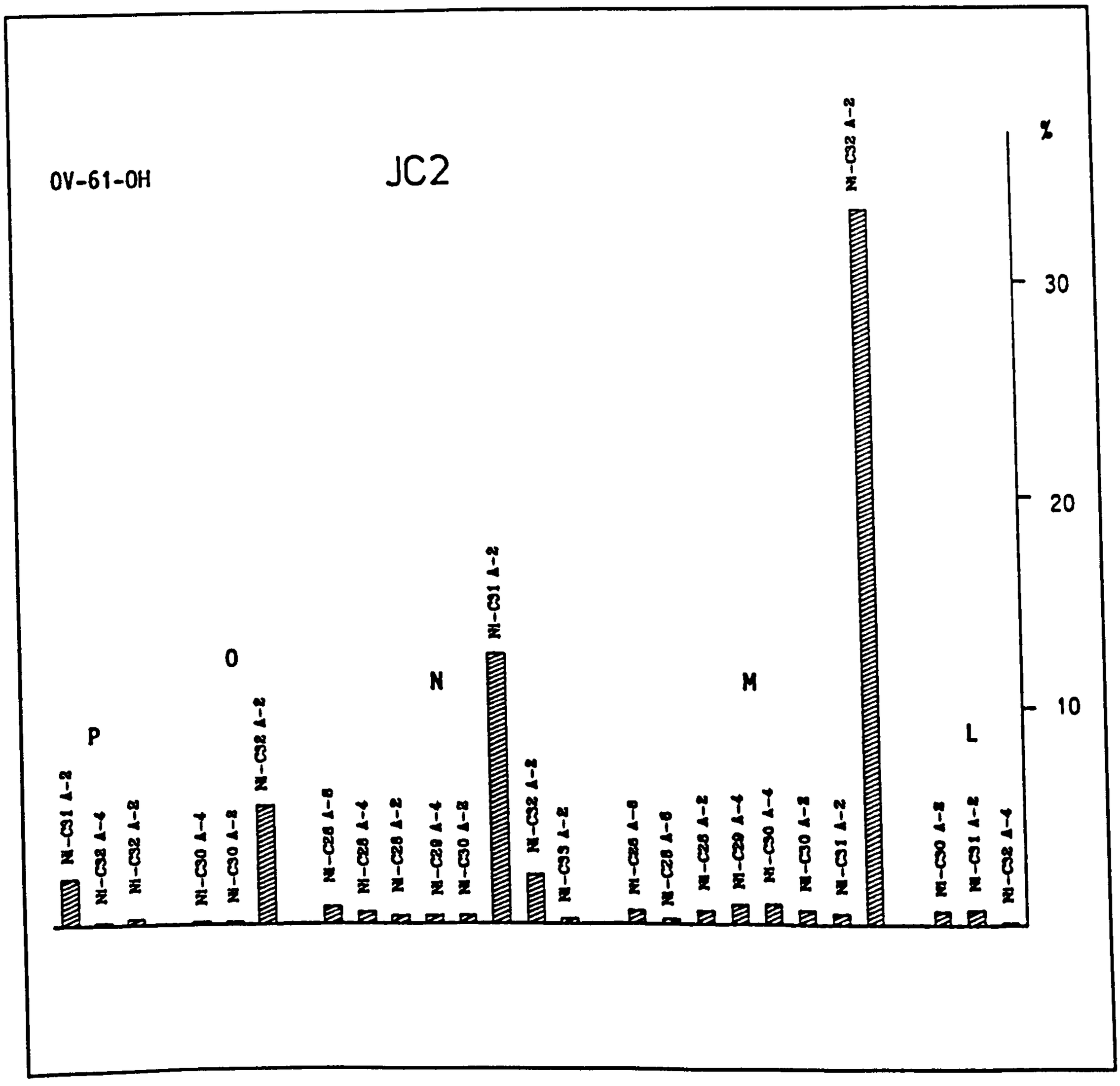


Fig.58 Graphic presentation of the nickel porphyrin distribution of GC-peaks L-P of JC2, analysed on an OV-61-OH coated column. See Fig.53.

V.2.1.3.1.3. GC/CIMS Analysis of Fraction JC2

The nickel porphyrin distribution deduced from EI-spectra is partially corroborated by chemical ionisation measurements of JC2. The two CI/NH_3-TIC chromatograms in Fig.60 and 61, carried out on the same columns used in the foregoing section show again the enhanced selectivity of the OV-61-OH coated column, but also broadened GC-peaks on both capillaries.
Moreover, in comparison to EI, in CI-mode the bleeding behaviour of the stationary phases at elevated temperatures has to be taken into account more seriously. Quasi-molecular ions of polycyclosiloxanes, formed by degradation of the stationary phases and commonly termed "bleeding", appear more intense at higher masses in CI, thus interfering with the molecular ion determination of metalloporphyrins. In this respect, PS-090 is superior to OV-61-OH and more sensitive owing to an improved signal to noise ratio.

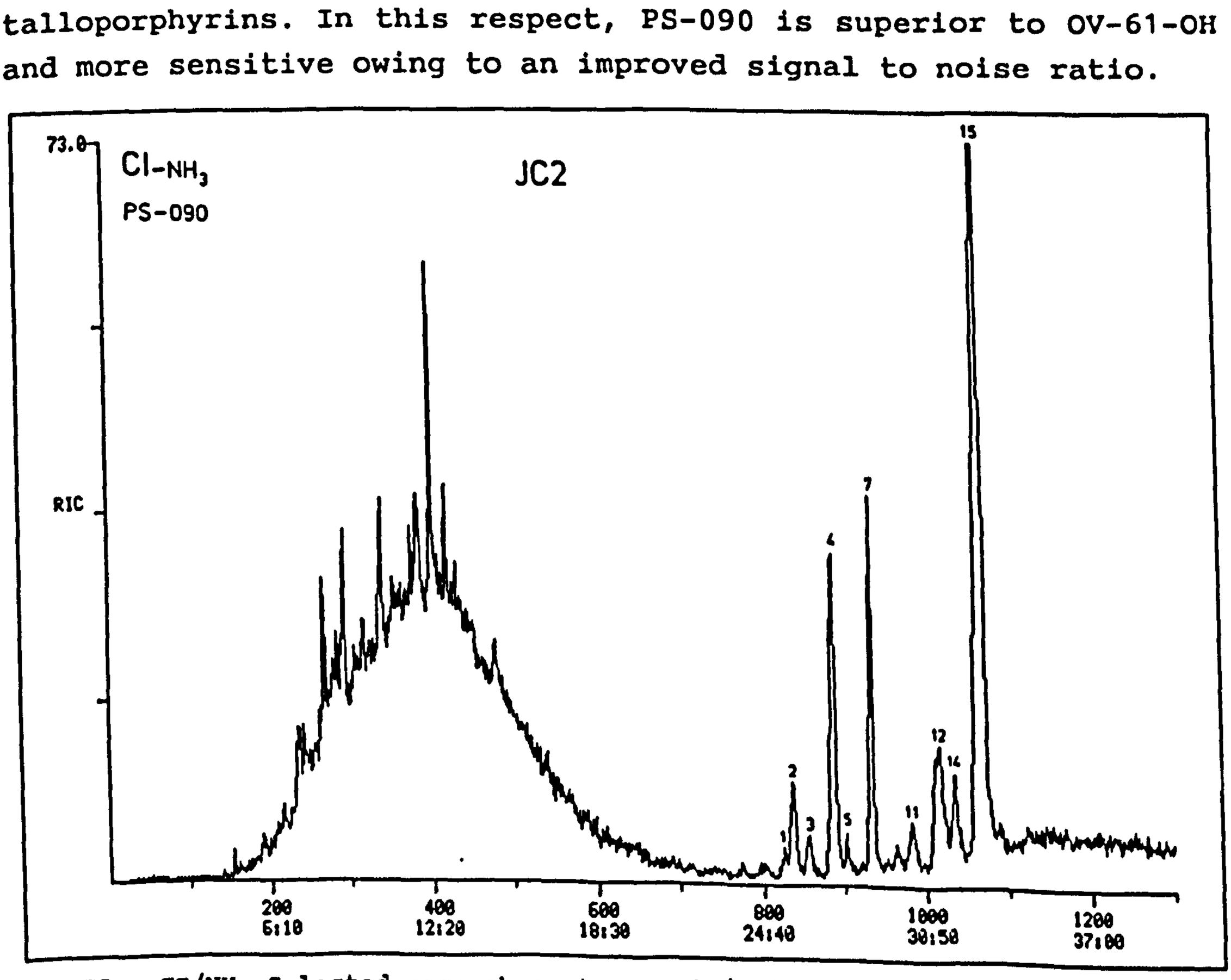


Fig.59 CI/NH_3 Selected mass chromatogram (m/z 400-650) of JC2 on a PS-090 coated column.

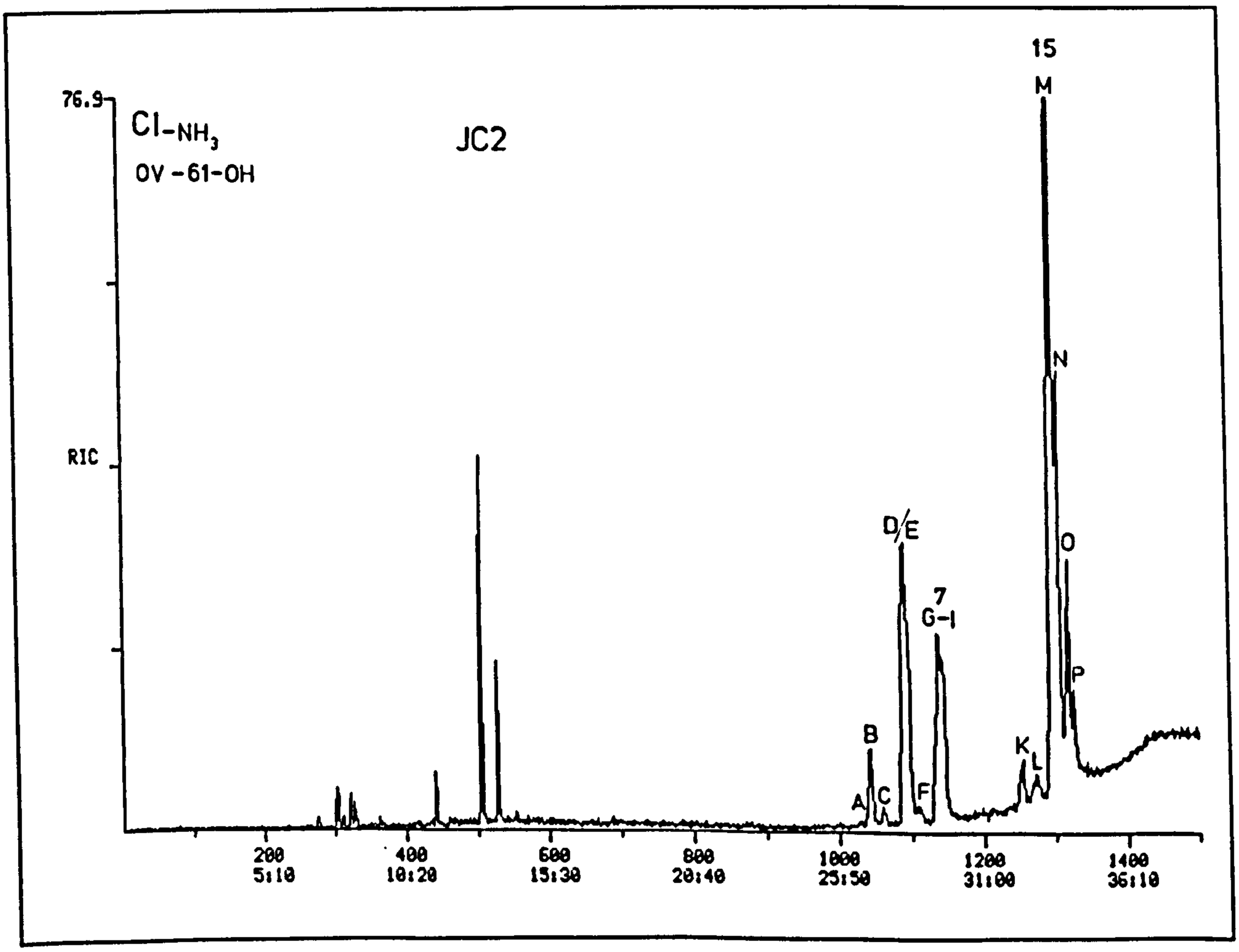


Fig.60 CI/NH_3 Selected mass chromatogram (m/z 450-650) of JC2 on an OV-61-OH coated column.

As emphasised in Section IV.2.2.1., fragmentation of substituents attached to the pyrrolic rings is not observed in CI-spectra, and at a first glance, unquestionable correlation of single molecular-ions should therefore be feasible. But, in chemical ionisation other correlation problems come into play. Owing to the protonation of tetrapyrroles, the ^{60}Ni-isotope peaks of the single metalloporphyrin quasi-molecular ions are too intense, leading to the wrong assumption that the GC peaks contains metalloporphyrins of lower degree of unsaturation (two, four or six masses higher), in addition to unambiguously assigned nickel porphyrin parent ions. This is demonstrated in the summed mass spectra of GC peak-15 (Fig.61) taken from the TIC-chromatogram of Fig.60 and of GC-peak 7a-c (Figs.62a-c) taken from Fig.60.

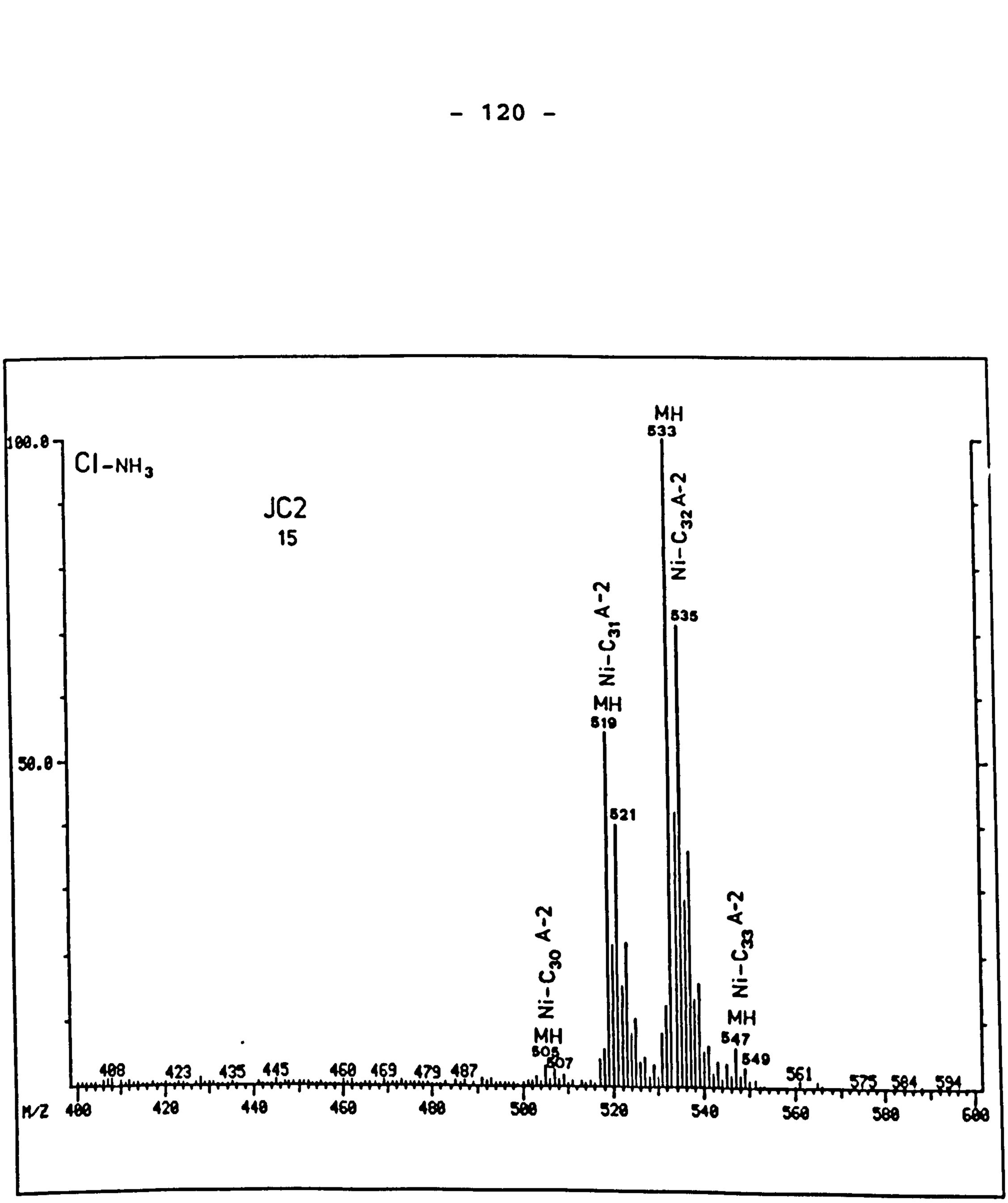
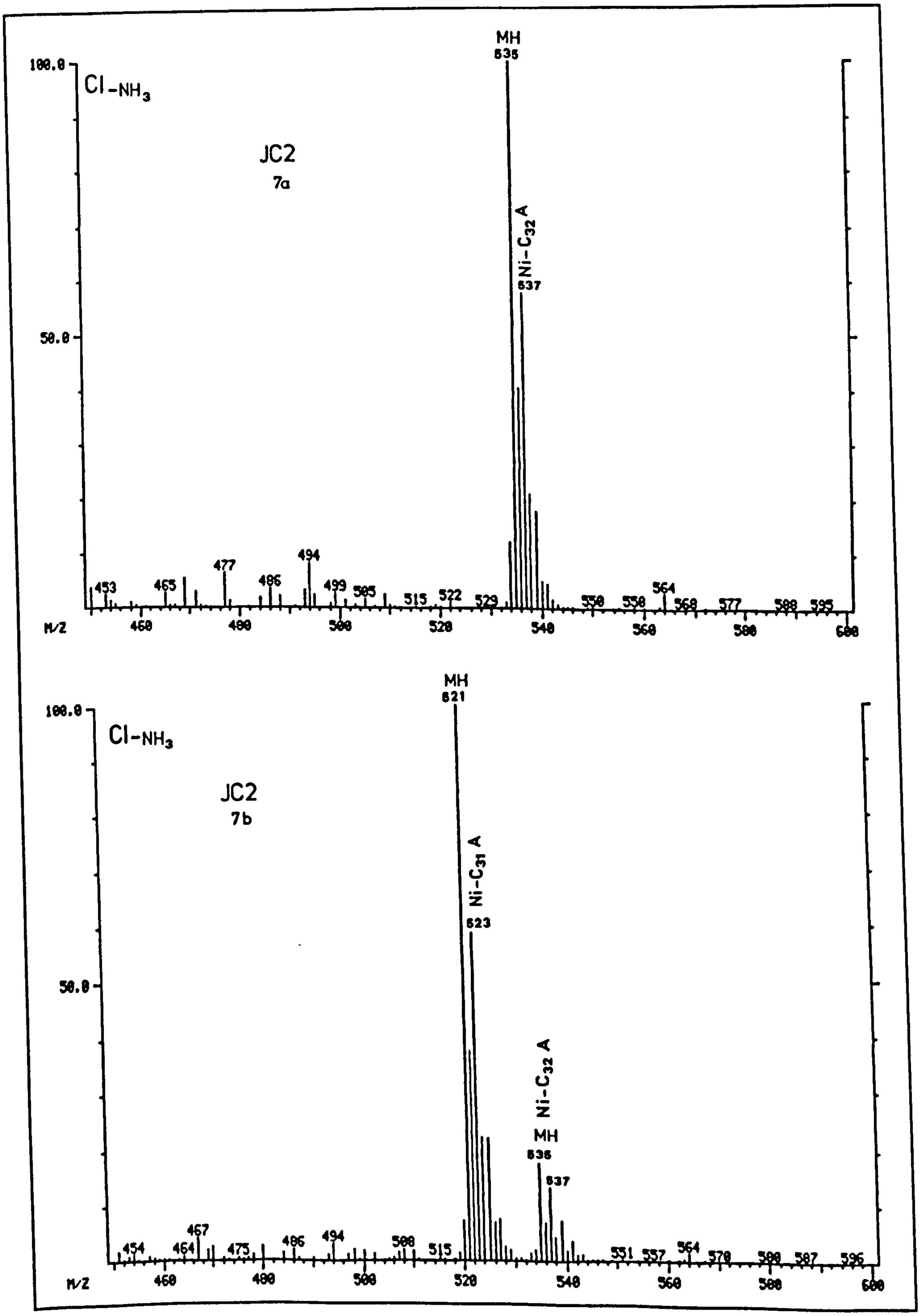


Fig.61 CI/NH_3 summed mass spectrum of scan 1291-1303 (peak 15) from TIC chromatogram shown in Fig.60.


100.0
Cl-NH3
JC2
7a
MH
535
Ni-C32 A
537
50.0
453
465
477
486
494
499
505
515
522
529
550
558
564
568
577
588
595
M/Z
460
480
500
520
540
560
580
600
100.0
Cl-NH3
JC2
7b
MH
621
Ni-C31 A
623
Ni-C32 A
MH
636
637
50.0
454
464
467
475
486
494
508
515
551
557
564
578
588
597
M/Z
460
480
500
520
540
560
580
600

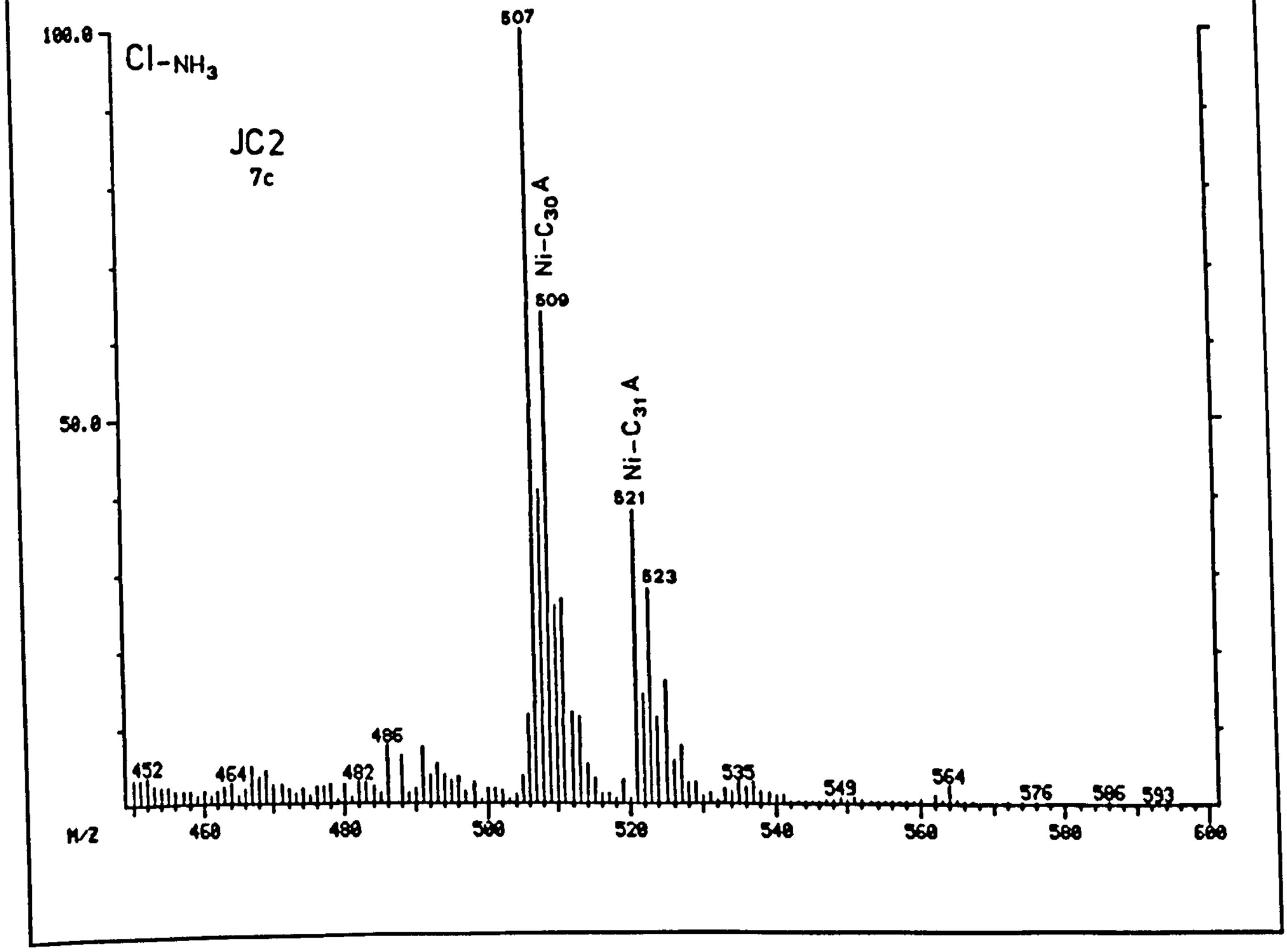


Fig.62 a-c CI/NH_3 summed mass spectrum of scan 1130-1134 (peak 7a), scan 1135-1141 (peak 7b) and scan 1143-1149 (peak 7c) from TIC chromatogram shown in Fig.60.

The pronounced formation of monopyrrolic fragments in CI, additionally depending on the nature of the central atom (see also Fig.23a-f), reduces the relative intensities of the quasi-molecular ions. Trace components appear therefore less abundant than in EI. Moreover, the unavoidable secondary reaction in the ion-source influences the chromatographic resolution (see Section IV. 2.4), which is already reduced due to peak broadening by adsorption in the interface line.

Therefore, in order to confirm the assignment of molecular-ions in nickel petroporphyrin extracts, chemical ionisation is only recommended as a supplement rather than a substitute for the more sensitive and, at least, more informative EI-measurements.

V.2.1.3.2. Semi-Systematic Characterisation of Fraction JC3

The second coloured liquid chromatographic fraction indicated by UV-absorption at 409 nm is fraction 18, in the following termed JC3. The FID chromatogram on the apolar, PS-347.5 coated column in Fig.31 exhibits 5 separated peaks, and a similar separation was obtained on a PS-090 column.
As shown in Fig.63, in comparison to fraction JC2 (see Fig.35a), all porphyrins of JC3 appear at higher retention temperatures. In spite of the fact, that the adsorption on the interface line is alike, the total ion chromatogram in Fig.64 shows nearly the same resolution.

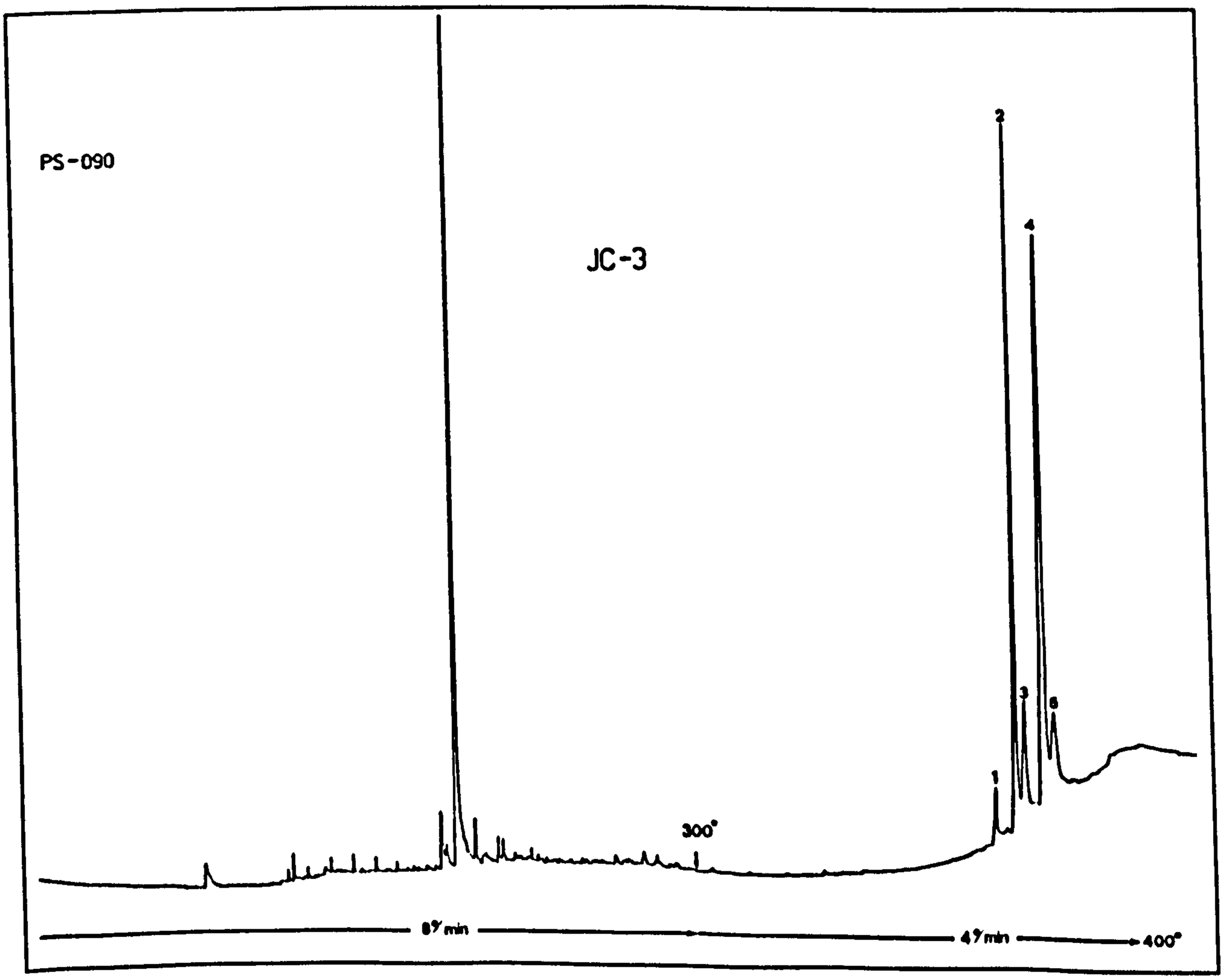


Fig.63 FID chromatogram of fraction JC3 on a PS-090 coated column

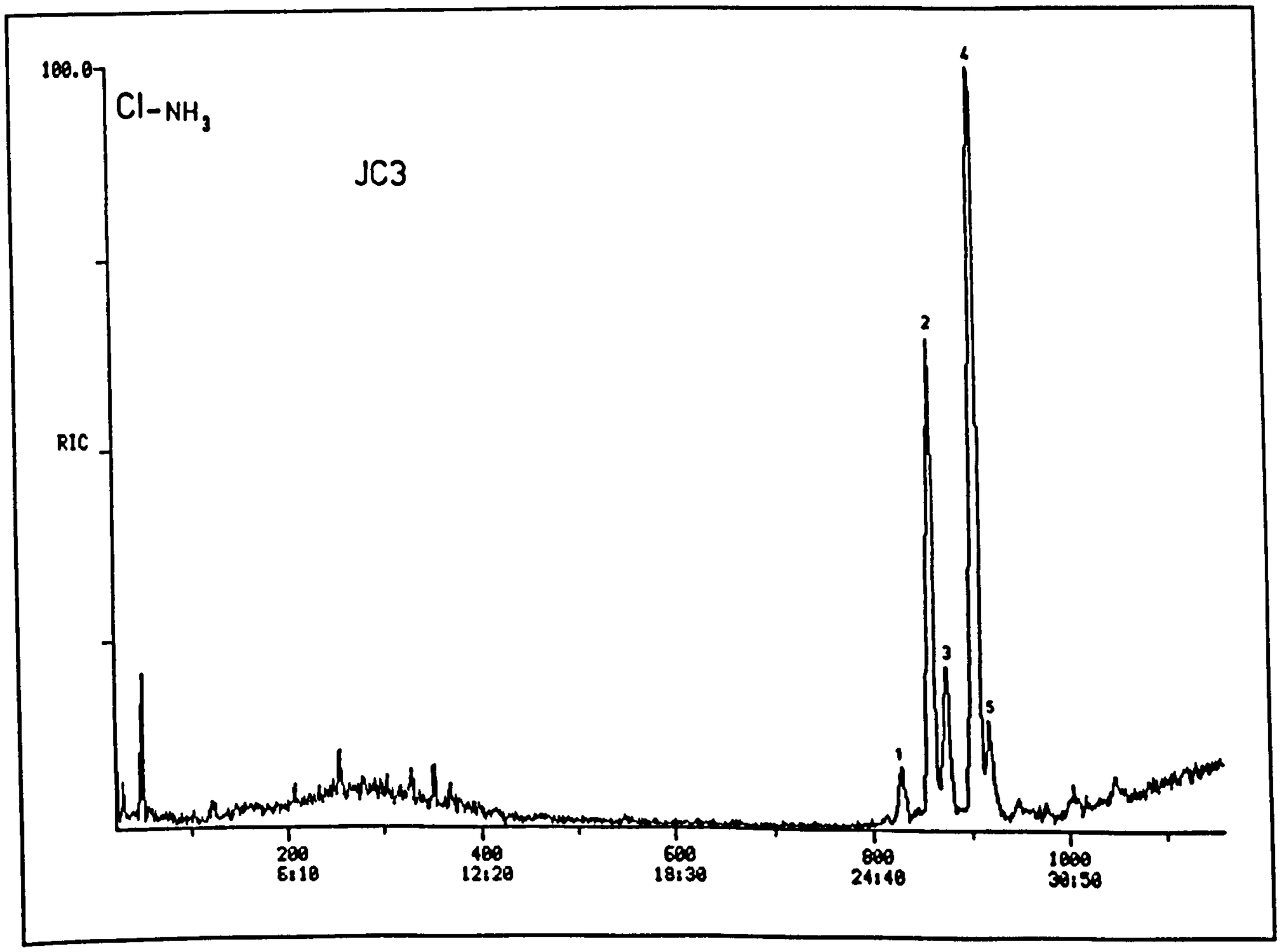


Fig.64 CI/NH_3 TIC chromatogram of fraction JC3 on a PS-090 coated column. For experimental details see Section VII.2.1.-2.2..

The ICPMS results in Fig 65 indicates that fraction JC3 predominantly contains vanadium as complexing metal moiety (97.4%) and other transition metals are of subordinate importance.
In contrast to nickel petroporphyrins, CI data of vanadyl porphyrins can unambiguosly be used for the assignment of respective quasi-molecular ions. The summed CI-NH_3 mass spectra of GC peak 1-5 in Figs.66-70, derived from the TIC chromatogram in Fig. 64, are in accordance with the ICP findings.

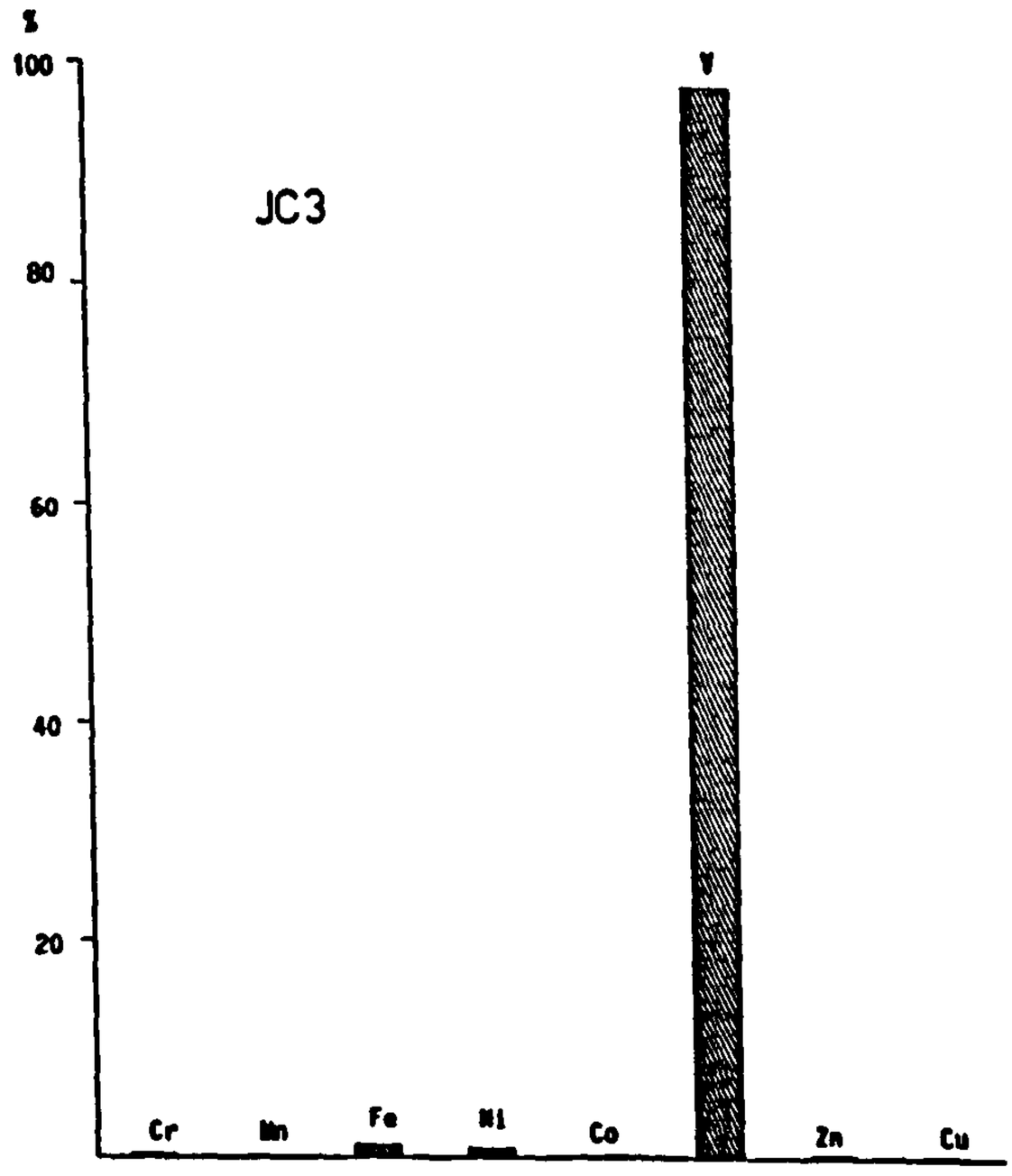


Fig.65 Graphical presentation of the metal distribution of fraction JC3 measured by ICPMS.

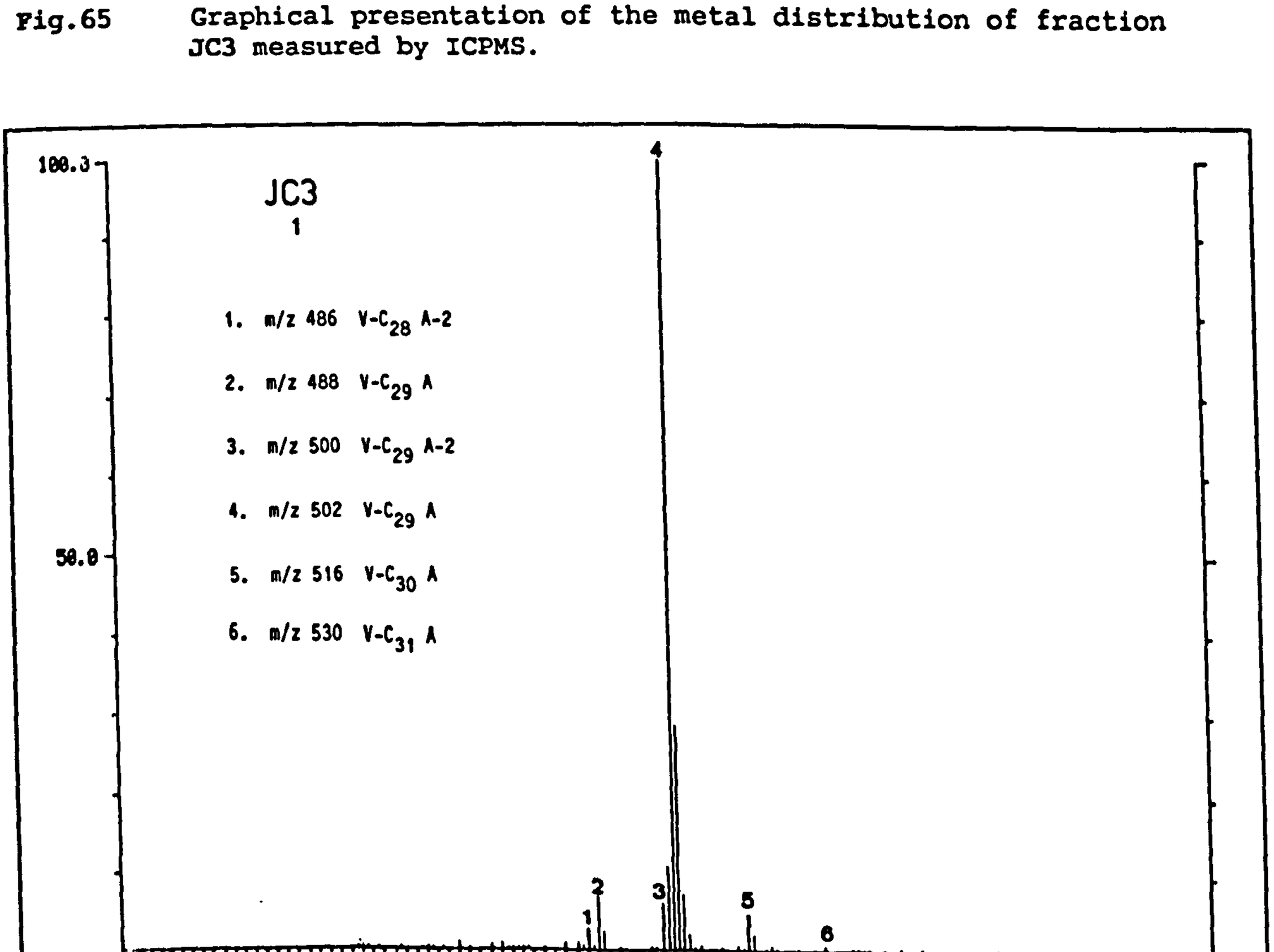


Fig.66 Summed CI/NH_3 mass spectrum of scans 826-834 (peak 1) from TIC chromatogram of fraction JC3 shown in Fig 64.

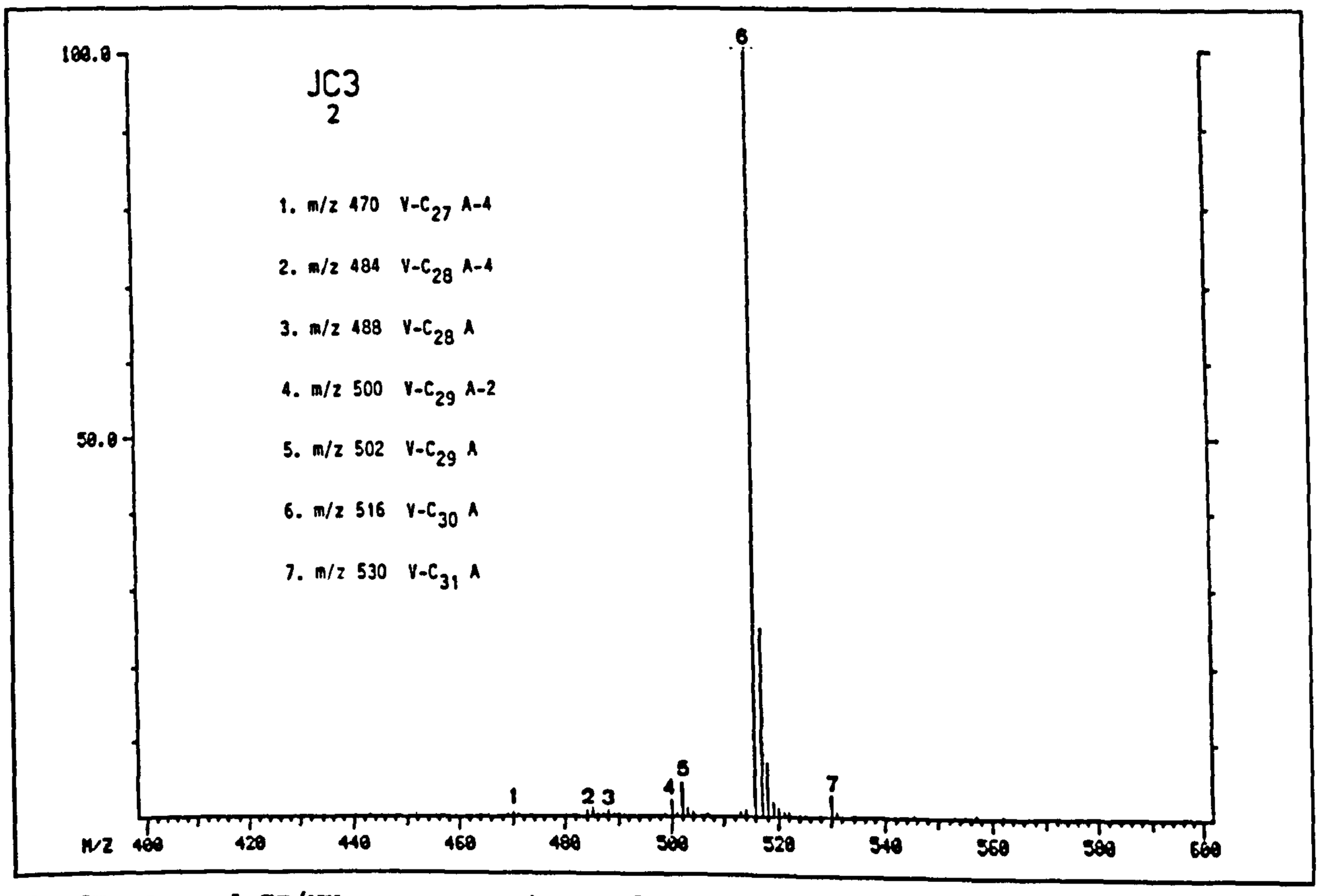


Fig.67 Summed CI/NH_3 mass spectrum of scans 854-865 (peak 2) from TIC chromatogram of fraction JC3 shown in Fig.64

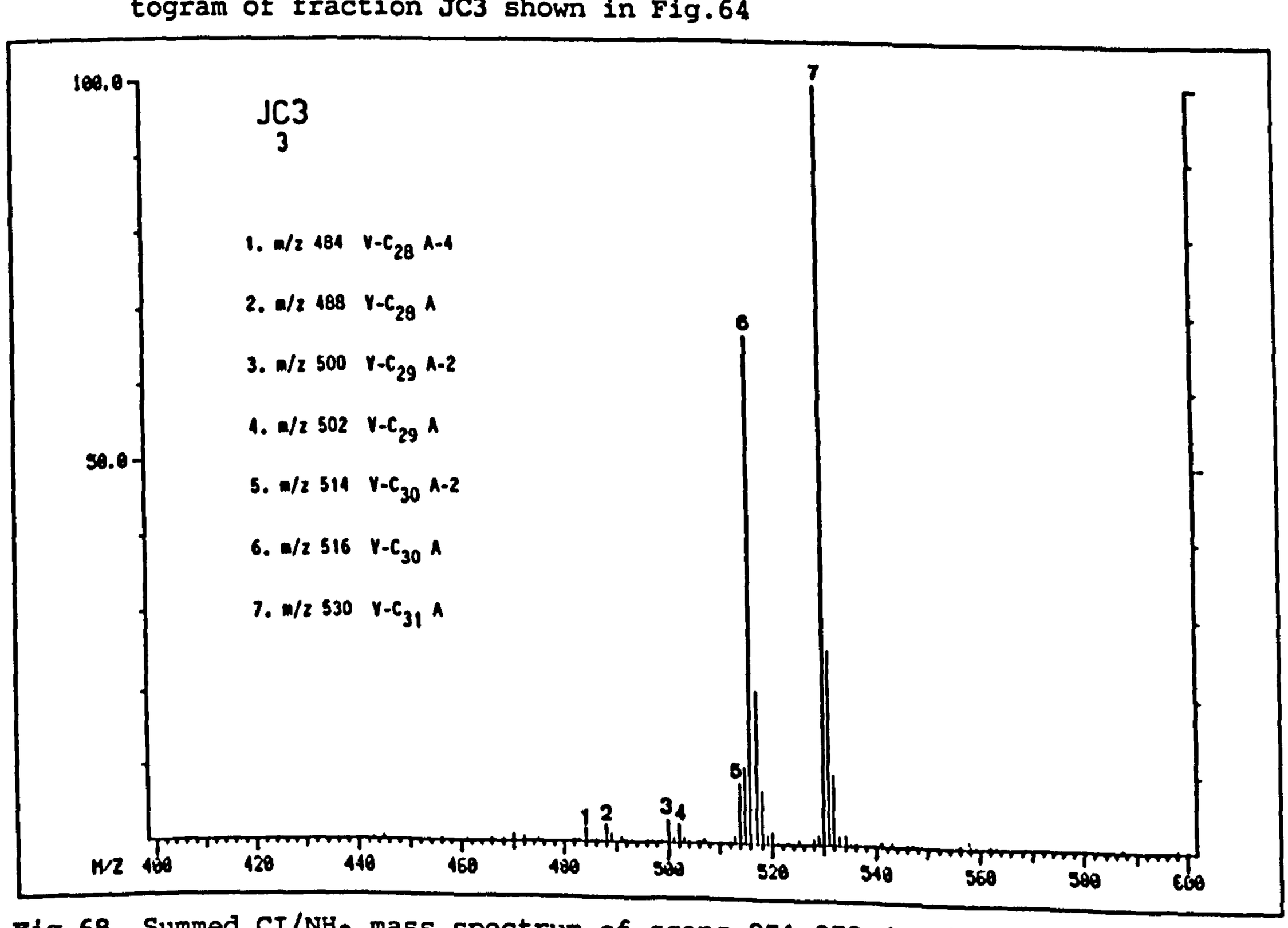


Fig.68 Summed CI/NH_3 mass spectrum of scans 871-879 (peak 3) from TIC chromatogram of fraction JC3 shown in Fig. 64.

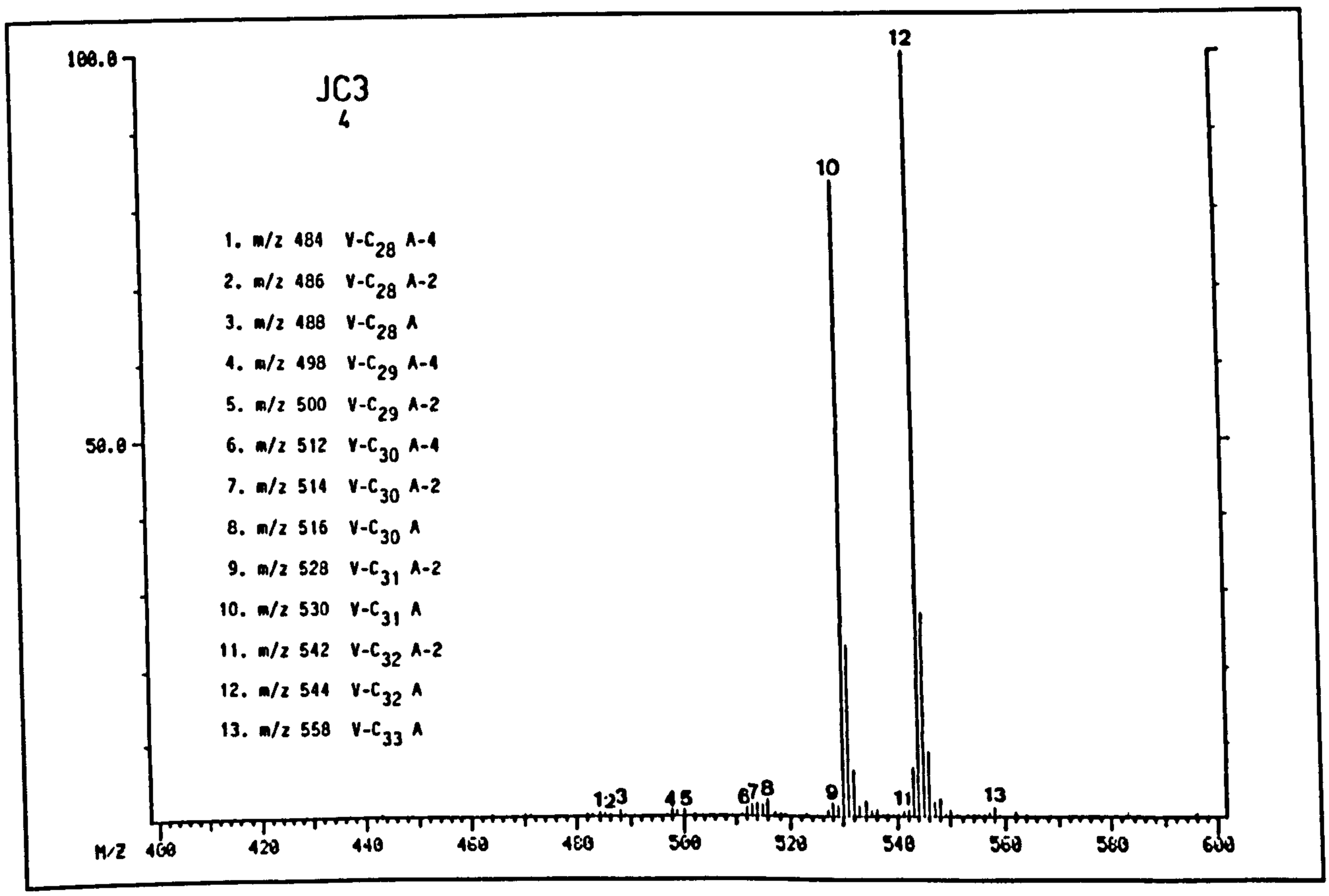


Fig.69 Summed CI/NH_3 mass spectrum of scans 899-911 (peak 4) from TIC chromatogram of fraction JC3 shown in Fig.64.

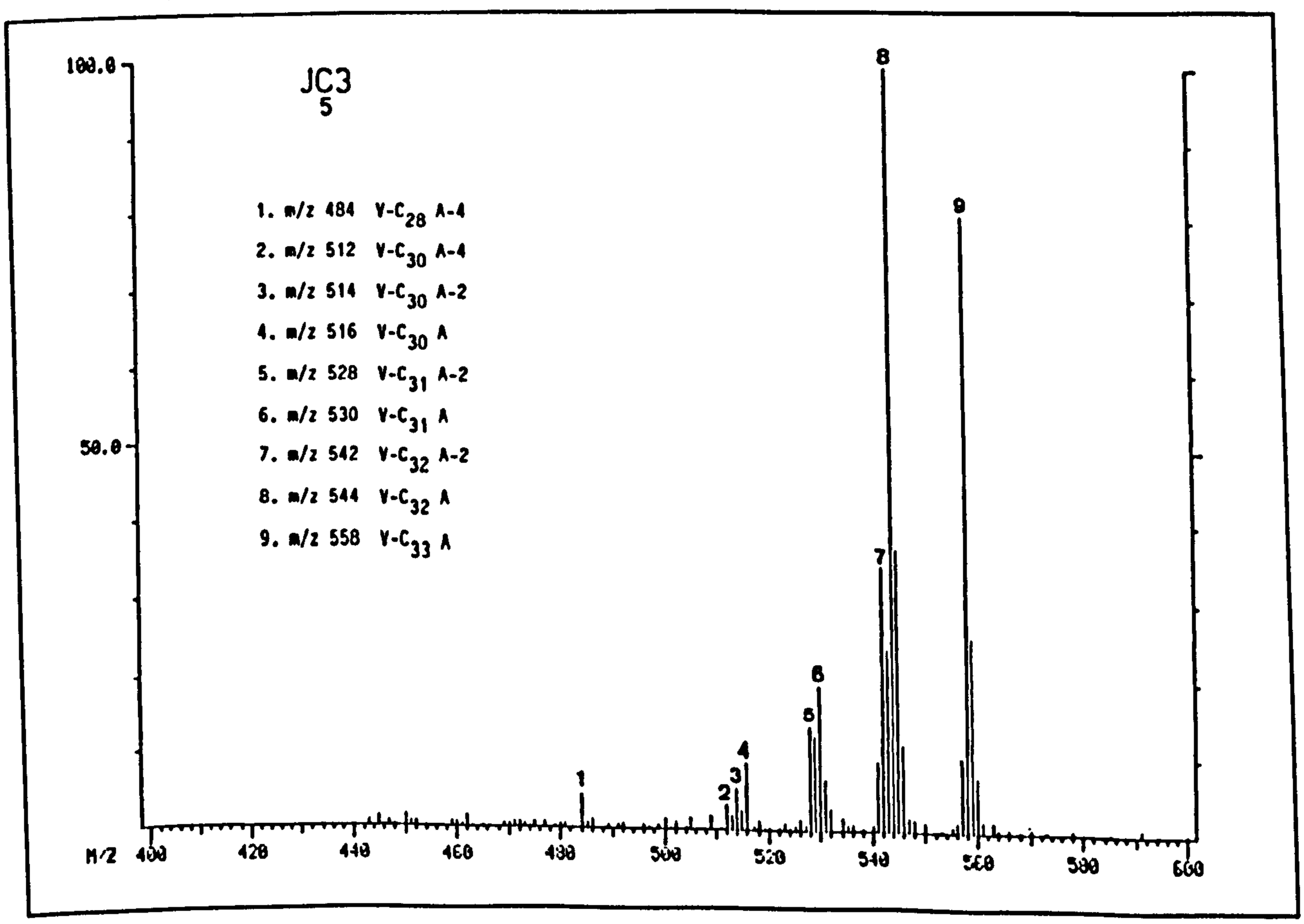


Fig.70 Summed CI/NH_3 mass spectrum of scans 917-923 (peak 5) from TIC chromatogram of fraction JC3 shown in Fig.64.

Owing to comparably simple isotope pattern of vanadyl-porphyrins, the semi-systematic characterisation is straightforward compared to nickel porphyrins, as demonstrated in the above spectra. Quantification was carried out on the absolute ion intensities, the contribution of ^{13}C-isotope peaks was taken into account. The results are presented in table 4 and the graph in Fig.71.

Table 4

GC peak No.	Quasi-Molecular Ion Distribution	Semi-Systematic Characterisation
1	1. m/z 486 2. m/z 488 3. m/z 500 4. m/z 502 5. m/z 516	V-C28 A-2 (0.07 %) V-C_{28} A (0.2 %) V-C_{29} A-2 (0.18 %) V-C_{29} A (2.98 %) V-C_{30} A (0.14 %)
2	1. m/z 470 2. m/z 484 3. m/z 488 4. m/z 500 5. m/z 502 6. m/z 516 7. m/z 530	V-C_{27} A-4 (0.12 %) V-C_{28} A-4 (0.18 %) V-C_{28} A (0.19 %) V-C_{29} A-2 (0.61 %) V-C_{29} A (1.19 %) V-C_{30} A (26.96%) V-C_{31} A (0.75 %)
3	1. m/z 484 2. m/z 488 3. m/z 500 4. m/z 502 5. m/z 514 6. m/z 516 7. m/z 530	V-C_{28} A-4 (0.1 %) V-C_{28} A (0.13 %) V-C_{29} A-2 (0.14 %) V-C_{29} A (0.14 %) V-C_{30} A-2 (0.39 %) V-C_{30} A (3.25 %) V-C_{31} A (4.87 %)

GC peak No.	Quasi-Molecular Ion Distribution	Semi-Systematic Characterisation
4	1. m/z 484	V-C_{28} A-4 (0.15 %)
	2. m/z 486	V-C_{28} A-2 (0.1 %)
	3. m/z 488	V-C_{28} A (0.24 %)
	4. m/z 498	V-C_{29} A-4 (0.24 %)
	5. m/z 500	V-C_{29} A-2 (0.25 %)
	6. m/z 512	V-C_{30} A-4 (0.26 %)
	7. m/z 514	V-C_{30} A-2 (0.38 %)
	8. m/z 516	V-C_{30} A (0.55 %)
	9. m/z 528	V-C_{31} A-2 (0.38 %)
	10. m/z 530	V-C_{31} A (22.86%)
	11. m/z 542	V-C_{32} A-2 (0.22 %)
	12. m/z 544	V-C_{32} A (34.83%)
	13. m/z 558	V-C_{33} A (0.27 %)
5	1. m/z 484	V-C_{28} A-4 (0.07 %)
	2. m/z 512	V-C_{30} A-4 (0.08 %)
	3. m/z 514	V-C_{30} A-2 (0.1 %)
	4. m/z 516	V-C_{30} A (0.14 %)
	5. m/z 528	V-C_{31} A-2 (0.22 %)
	6. m/z 530	V-C_{31} A (0.27 %)
	7. m/z 542	V-C_{32} A-2 (0.55 %)
	8. m/z 544	V-C_{32} A (1.48 %)
	9. m/z 558	V-C_{33} A (1.29 %)

Table 4: Vanadyl porphyrin distribution of fraction JC3, analysed on a PS-090 coated column.

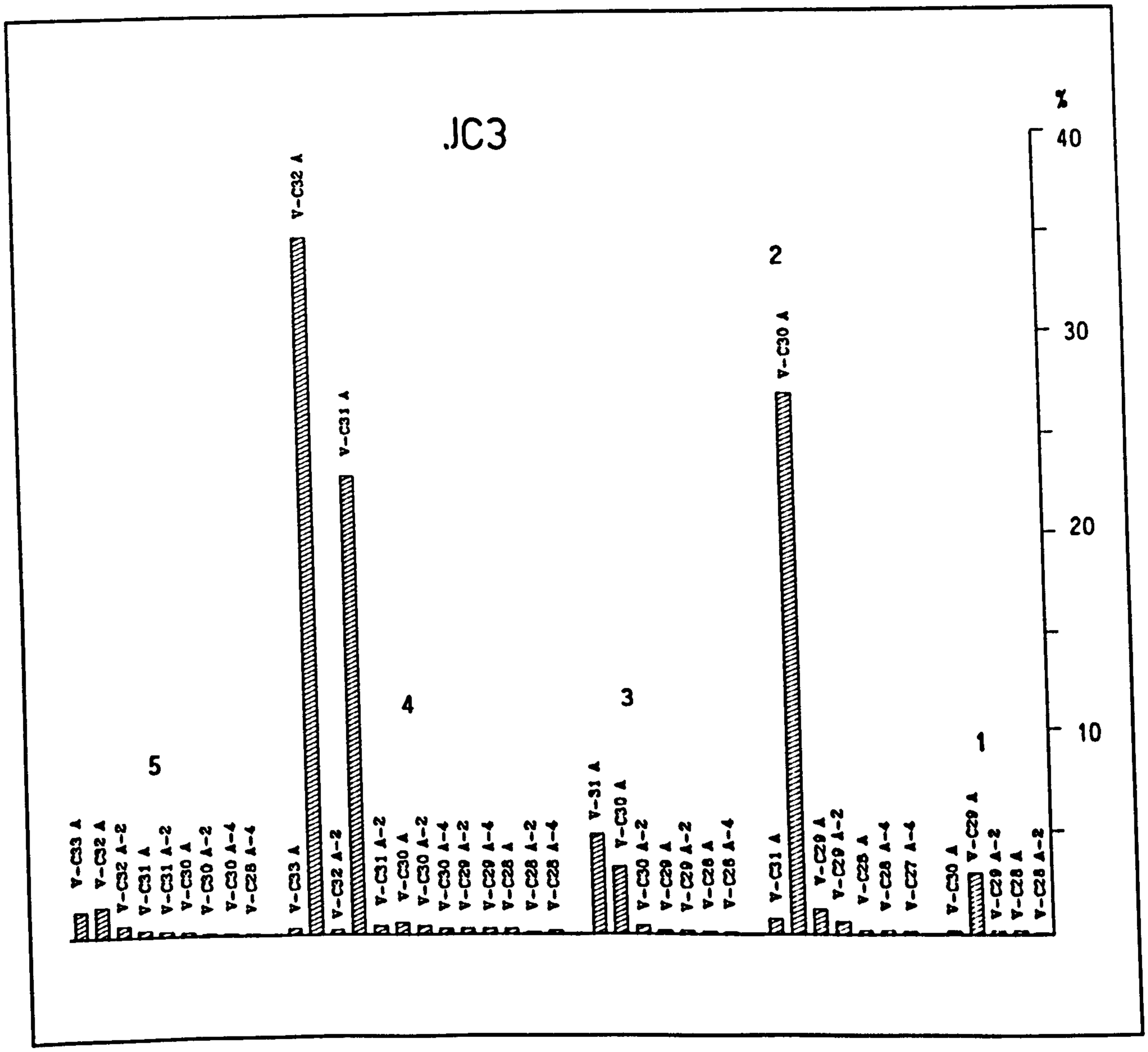


Fig.71 Graphic presentation of vanadyl porphyrin distribution of GC peaks 1-5 of JC3.

V.2.1.3.3. Semi-Systematic Characterisation of Fraction JC4 and JC5

The liquid chromatographic fraction 19, in separation scheme Table 2 termed JC4, represents the overlap of JC3 and JC5 (see also Fig.32). Since this sample contains no additional information it was not further examined by GC/MS.
The combined liquid chromatographic fractions 20-26, in the following termed JC5, contain vanadyl-porphyrins structurally different from those in JC3. The fraction has an UV absorption band at 409 nm and is quantitatively the most important sample, although the FID trace exhibits only 8 separated or nearly separated peaks. The gas chromatographic pattern of the porphyrin section of JC5 is very similar to that of the crude extract shown in Fig.28.

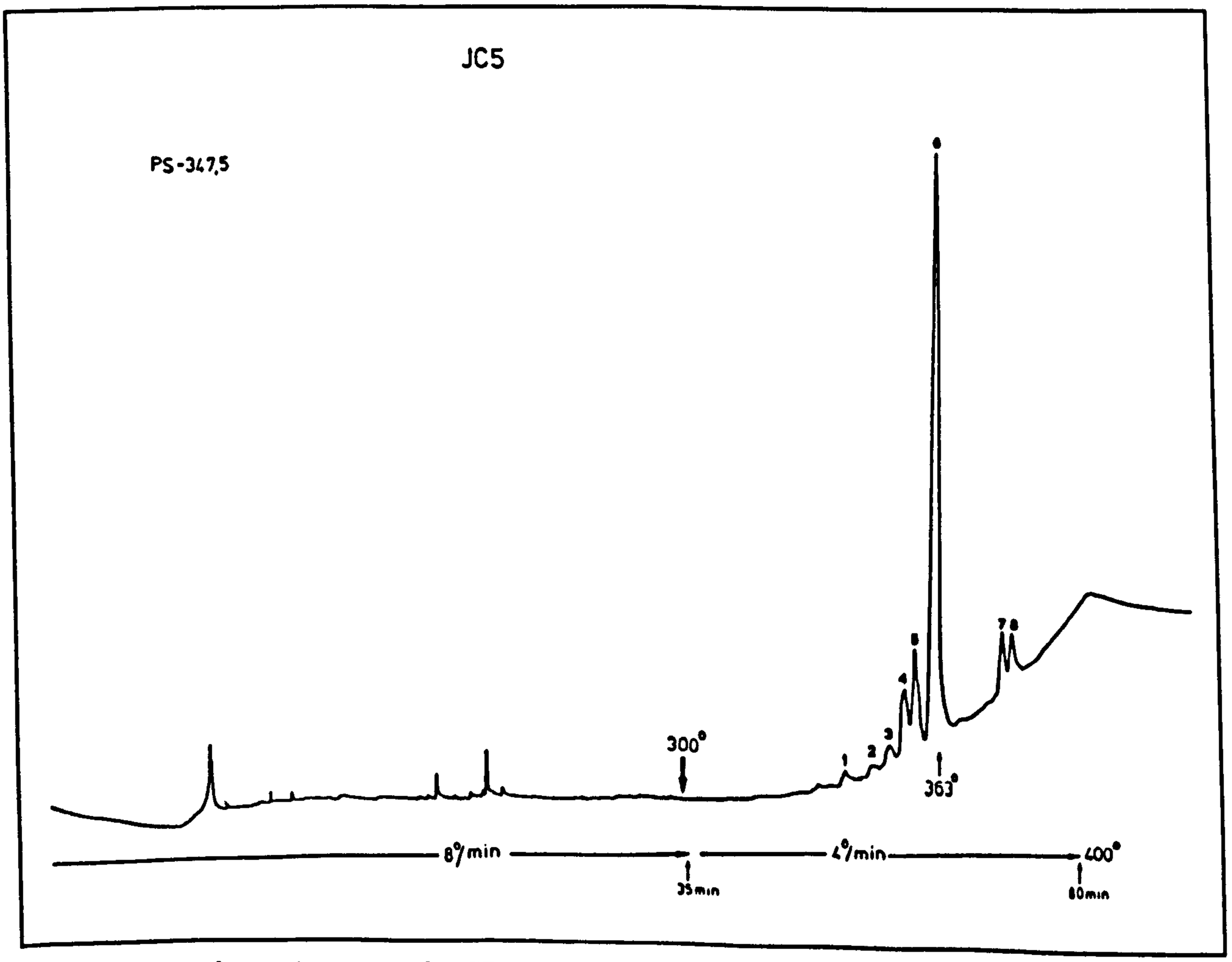


Fig.72 FID chromatogram of JC5 on a PS-347.5 coated capillary column.

The ICPMS finding shows that vanadium is again the most abundant chelating metal (99.17%) and that the amount of other transition metal elements is negligible.

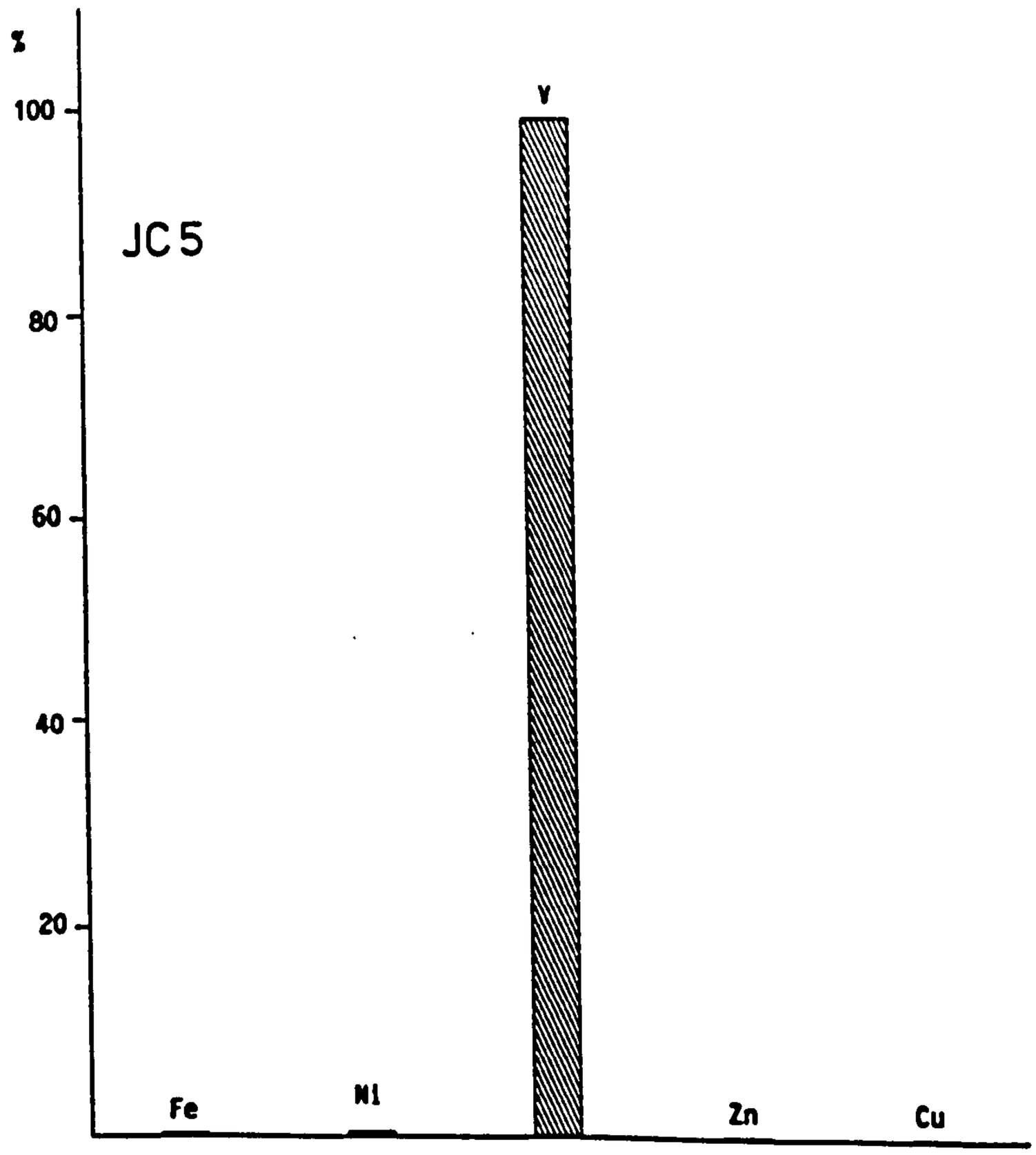


Fig.73 Graphical presentation of the metal distribution of fraction JC5 measured by ICPMS.

The CI/NH_3 selected mass chromatogram in Fig.74 demonstrates that the resolution of the FID chromatogram could not be maintained and that peak broadening, obviously directly dependent on the retention temperature, is more pronounced than in JC2 and JC3.

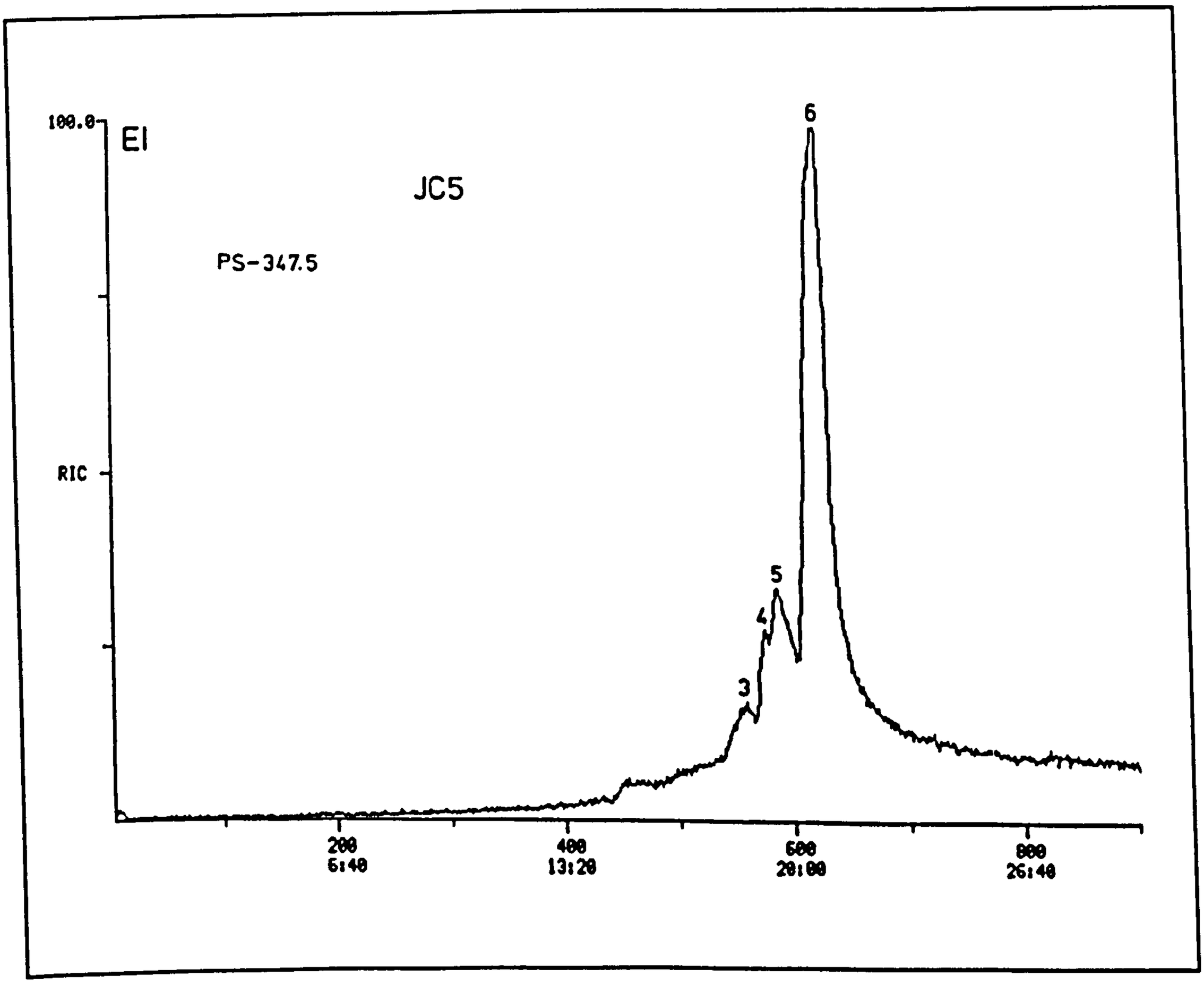


Fig.74 Selected mass chromatogram (m/z 400-600) of fraction JC5 on a PS-347.5 coated column.

The summed CI-NH_3 mass spectra presented in Fig.75-77 are again in accordance with the ICPMS results. They exhibits that the most abundant vanadylporphyrins in JC5 are more highly unsaturated than in JC3, and they confirm again the direct correlation between the degree of unsaturation and high retention temperatures.

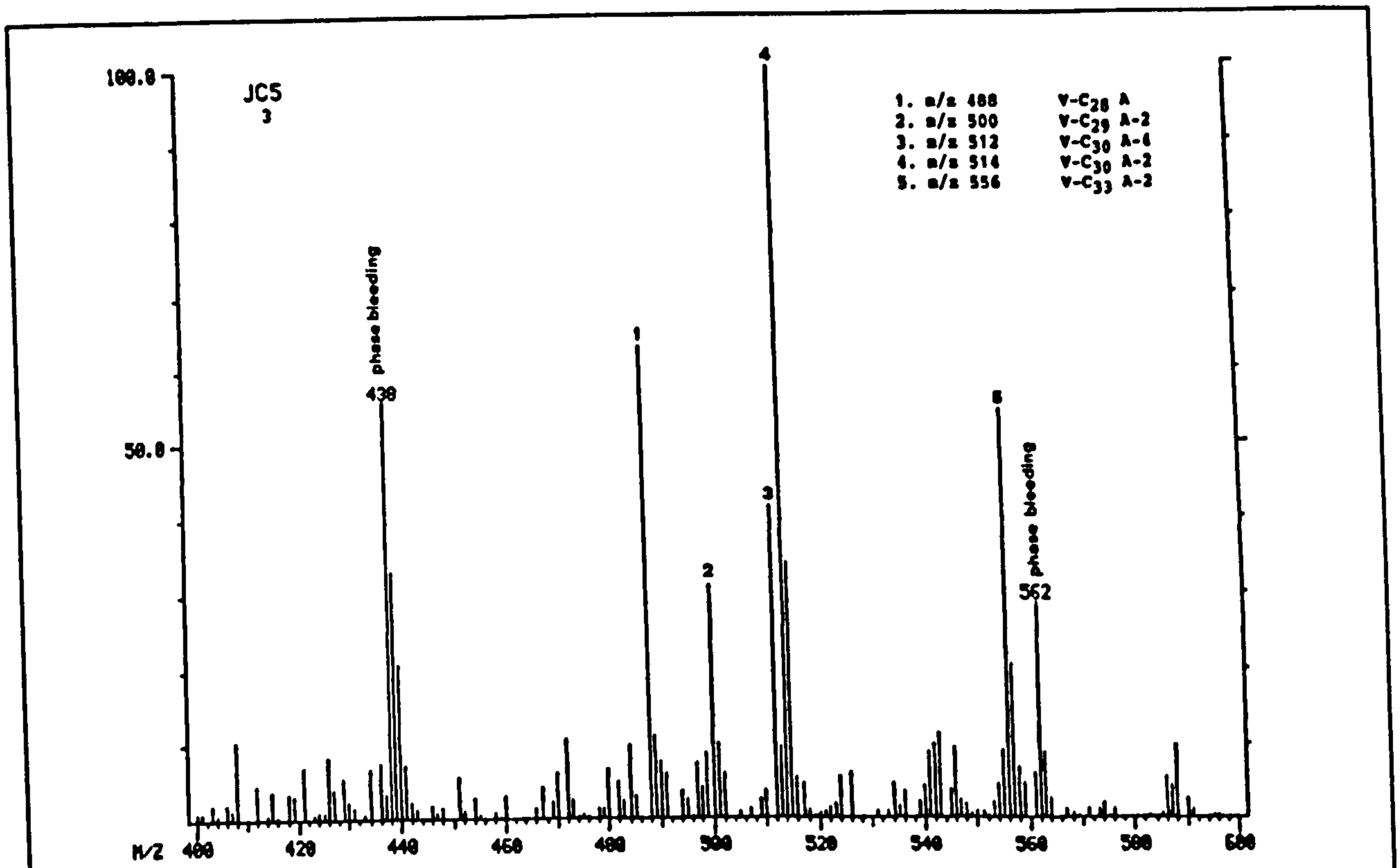


Fig.75 Summed CI/NH_3 mass spectrum of scans 539-564 (peak 3) from mass chromatogram of fraction JC5 shown in Fig.72.

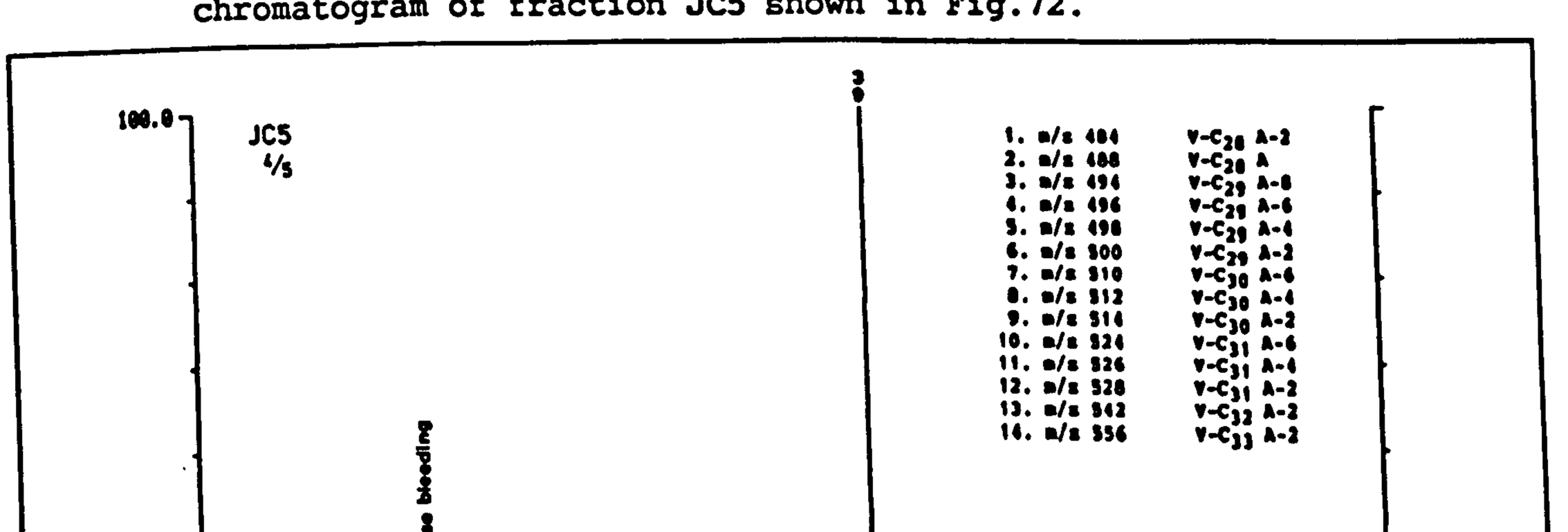


Fig.76 Summed CI/CH_3 mass spectrum of scans 566-605 (peak 4-5) from mass chromatogram of fraction JC5 shown in Fig,72

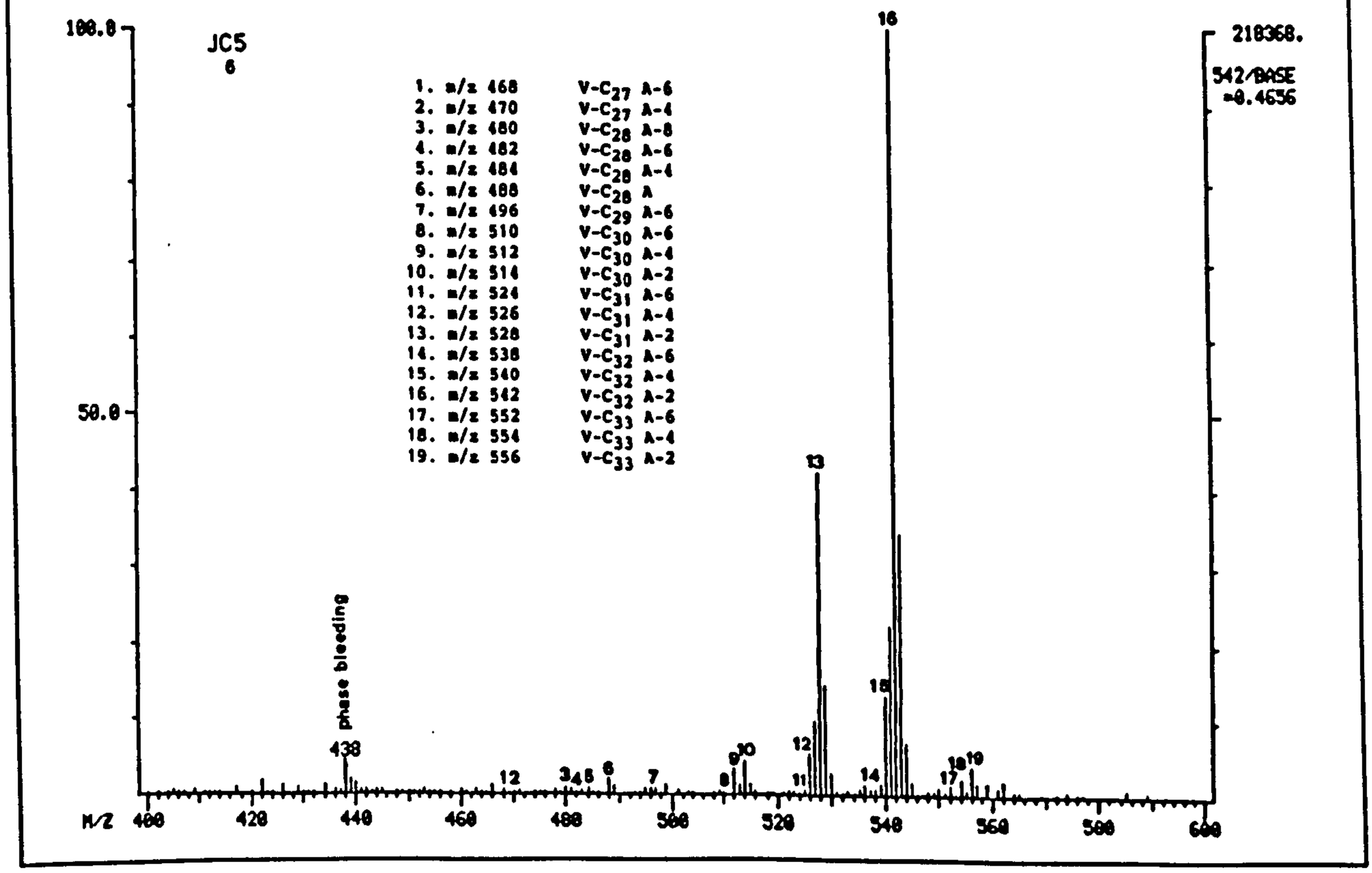


Fig.77 Summed CI/NH_3 mass spectrum of scans 607-635 (peak 6) from mass chromatogram of fraction JC5 shown in Fig.74.

Quantification was carried out, as for JC2 and JC3 by the absolute ion intensities, taking the ^{13}C-isotope contributions into consideration. The results are presented in Table 5 and the graph in Fig.78.

Table 5

GC peak No	Quasi- Molecular Ion Distribution	Semi-Systematic Characterisation
3	1. m/z 488	$V\text{-}C_{28}$ A (0.43 %)
	2. m/z 500	$V\text{-}C_{29}$ A-2 (0.45 %)
	3. m/z 512	$V\text{-}C_{30}$ A-4 (0.44 %)
	4. m/z 514	$V\text{-}C_{30}$ A-2 (1.45 %)
	5. m/z 556	$V\text{-}C_{33}$ A-2 (0.76 %)

GC peak No	Quasi-Molecular Ion Distribution	Semi-Systematic Characterisation
4	1. m/z 500	V-C_{29} A-2 (0.16 %)
	2. m/z 512	V-C_{30} A-4 (0.33 %)
	3. m/z 514	V-C_{30} A-2 (5.16 %)
5	1. m/z 484	V-C_{28} A-2 (0.16 %)
	2. m/z 488	V-C_{28} A (1.21 %)
	3. m/z 494	V-C_{29} A-8 (0.14 %)
	4. m/z 496	V-C_{29} A-6 (0.23 %)
	5. m/z 498	V-C_{29} A-4 (0.28 %)
	6. m/z 500	V-C_{29} A-2 (0.7 %)
	7. m/z 510	V-C_{30} A-6 (0.14 %)
	8. m/z 512	V-C_{30} A-4 (2.04 %)
	9. m/z 514	V-C_{30} A-2 (7.53 %)
	10. m/z 524	V-C_{31} A-6 (0.14 %)
	11. m/z 526	V-C_{31} A-4 (0.88 %)
	12. m/z 528	V-C_{31} A-2 (5.57 %)
	13. m/z 542	V-C_{32} A-2 (0.31 %)
	14. m/z 556	V-C_{33} A-2 (0.31 %)
6	1. m/z 468	V-C_{27} A-6 (0.47 %)
	2. m/z 470	V-C_{27} A-4 (0.2 %)
	3. m/z 480	V-C_{28} A-8 (0.4 %)
	4. m/z 482	V-C_{28} A-6 (0.2 %)
	5. m/z 484	V-C_{28} A-4 (0.4 %)
	6. m/z 488	V-C_{28} A (0.44 %)
	7. m/z 496	V-C_{29} A-6 (0.24 %)
	8. m/z 510	V-C_{30} A-6 (0.19 %)
	9. m/z 512	V-C_{30} A-4 (0.62 %)
	10. m/z 514	V-C_{30} A-2 (0.93 %)
	11. m/z 524	V-C_{31} A-6 (0.28 %)
	12. m/z 526	V-C_{31} A-4 (1.8 %)
	13. m/z 528	V-C_{31} A-2 (16.07 %)
	14. m/z 538	V-C_{32} A-6 (0.31 %)
	15. m/z 540	V-C_{32} A-4 (5.09 %)
	16. m/z 542	V-C_{32} A-2 (40.74 %)
	17. m/z 552	V-C_{33} A-6 (0.62 %)
	18. m/z 554	V-C_{33} A-4 (0.89 %)
	19. m/z 556	V-C_{33} A-2 (1.01 %)

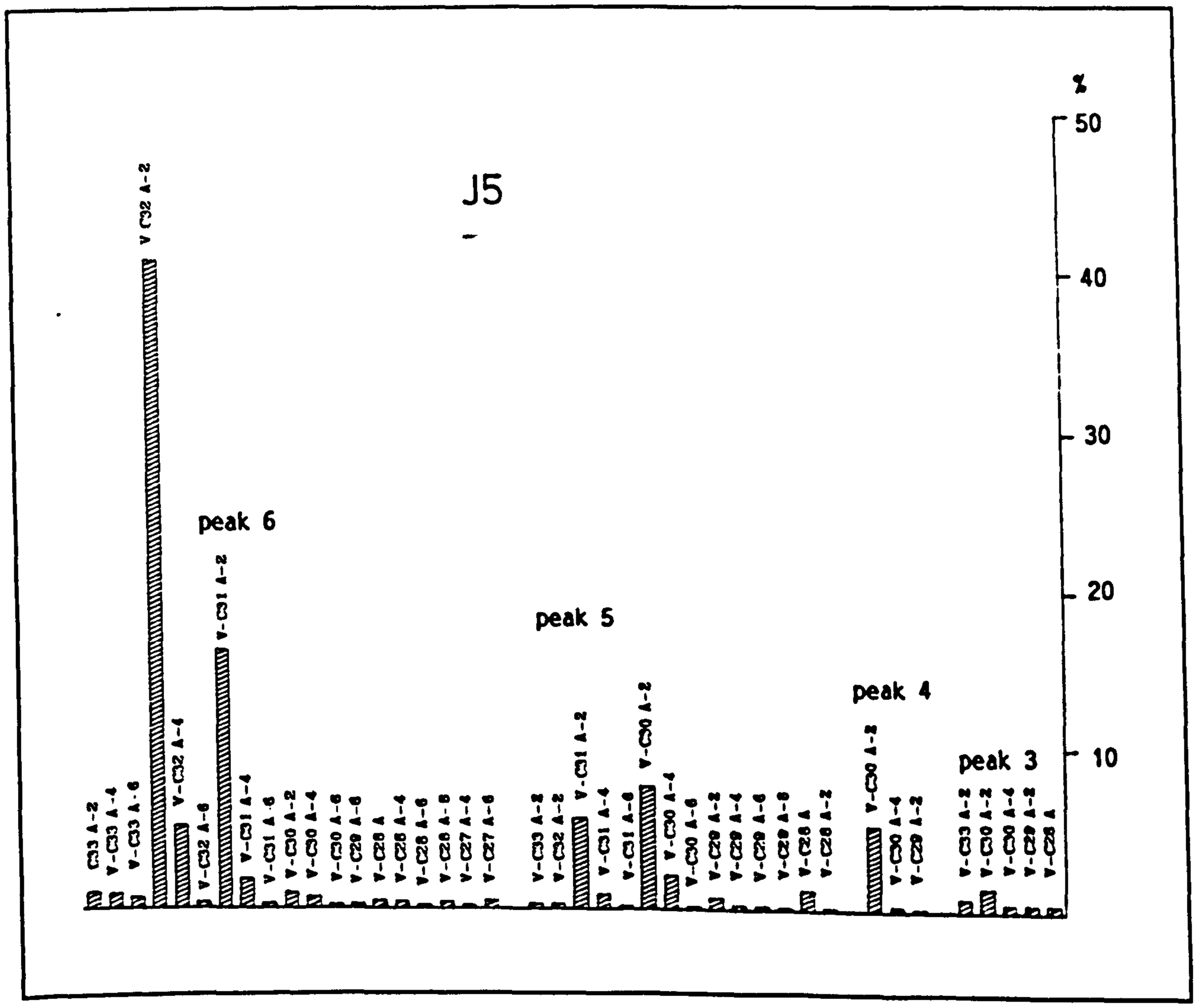


Fig.78 Graphic presentation of vanadyl-porphyrin distribution of GC peak 3, 4-5 and 6 of JC5.

With fraction JC5 the very limits of high temperature GC and GC/MS in the mixture analysis of petroporphyrins are reached. Gas chromatography failed in the analysis of the following two coloured column liquid chromatographic fractions JC6 to JC7 of Julia Creek oil shale. After several injections of these samples the entrance of the capillary columns is contaminated with nonvolatile residues but no signal in the porphyrin retention range could be observed, although from UV-absorbtion there is no doubt that these fractions contain components of porphyrin nature.

The comparison among JC2 to JC5 reveals quite clearly that the structural characteristics of a given porphyrin and the degree of unsaturation, rather than the molecular weight, are the limiting factors for the use of high temperature GC. This can be explained by the assumption, that not just the polarity of substituents but also the tendency for self-association determines the non-volatility of petroporphyrins. Some of them may be organised in larger units (trimers, tetramers etc.) as already discussed in Section I.1.1.

The liquid chromatographic fractions JC6-JC7 were therefore examined by means of capillary SFC/MS as logical extension of high temperature gas chromatography/MS.

V.2.2. Analysis of Demetallated Free Base Porphyrins of Fractions JC2, JC3 and JC5

In Section I.3.3.it was emphasised, that the best separation of free base petroporphyrins is obtained on an OV-61-OH coated column, but in the case of demetallated JC5, at the maximum working temperature of this stationary phase, the chromatogram is somewhat spoiled by an intensive column "bleeding".
In order to avoid the interference of polysiloxane degradation products with the interpretation of full porphyrin spectra, particularly in the CI-mode, the more temperature stable PS-090 was chosen as stationary phase to analyse the free bases of JC2, JC3 and JC5, respectively, at the cost of a slightly reduced selectivity. The FID chromatograms of the first two fractions are depicted in Figs.79-80.

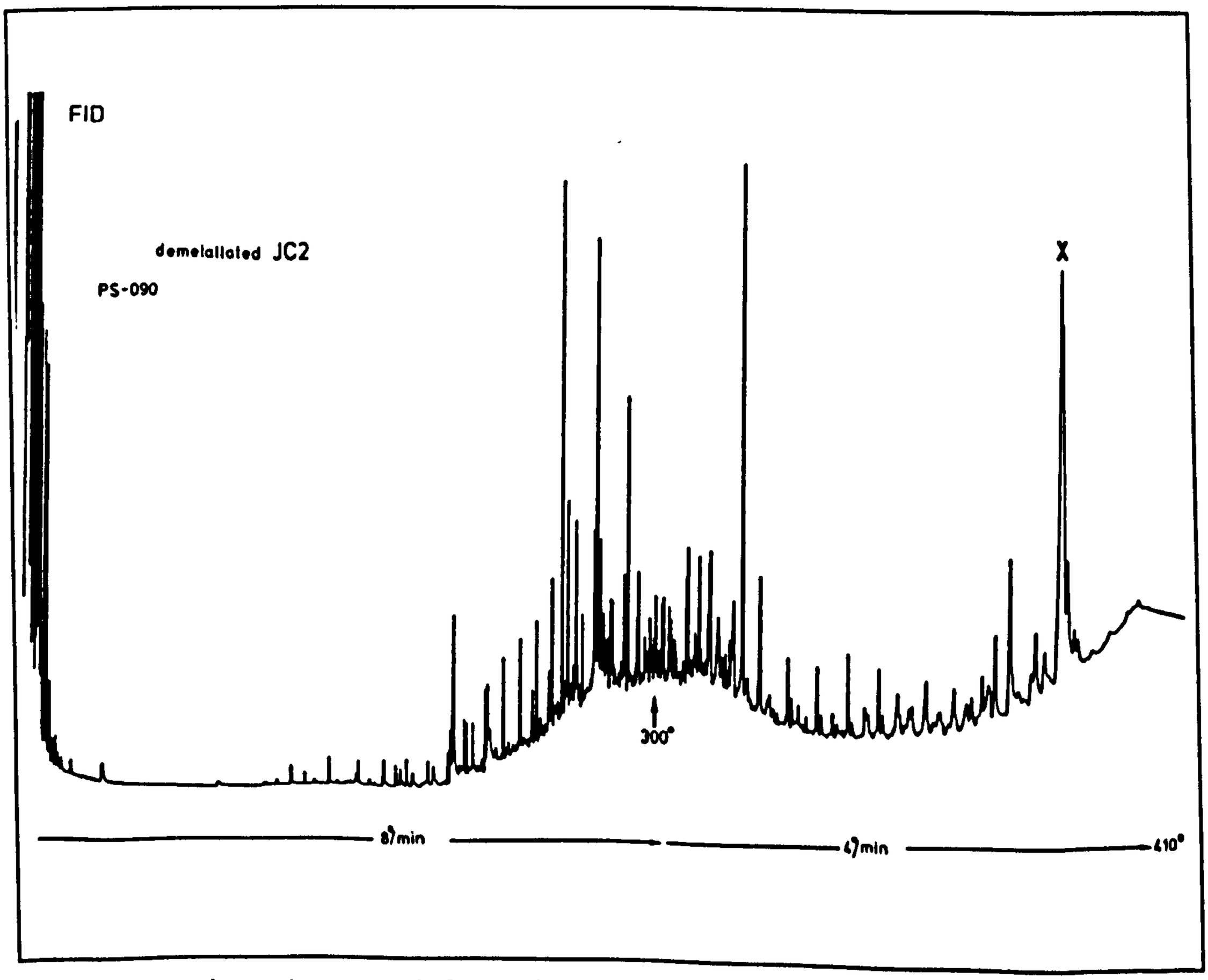


Fig.79 FID chromatogram of demetallated fraction JC2 (for X see Fig.95).

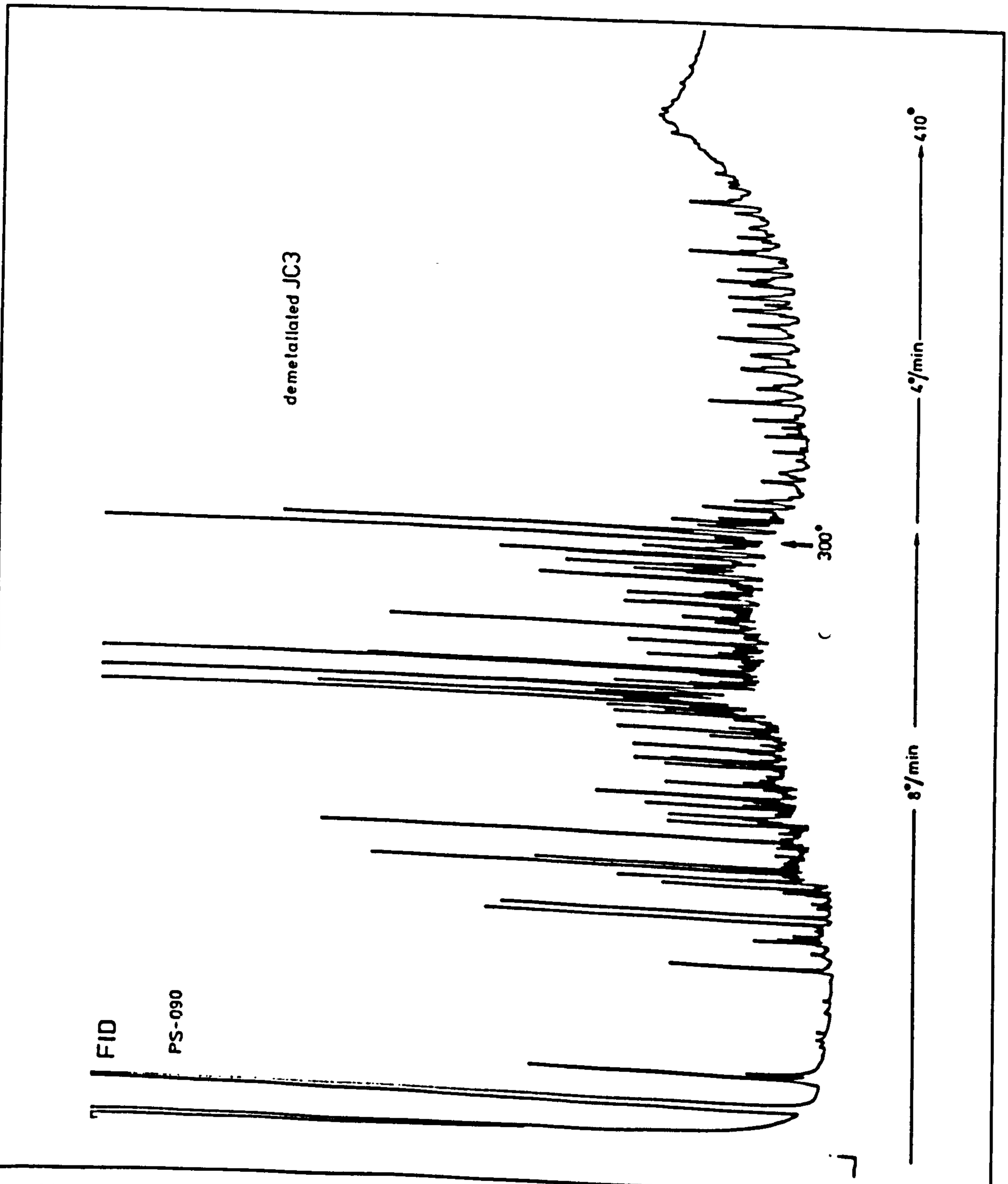


Fig.80 FID chromatogram of demetallated fraction JC3.

In spite of the fact that the upper temperature ranges of both foregoing FID chromatograms shows some peaks at retention temperatures expected for petroporphyrins, GC/MS measurements reveal that none of these components are free base porphyrins.
One may speculate about the disappearance of the free bases. The simplest explanation is given by the following circumstances:

This study was carried out with only 11g pulverised oil shale, from which 94.12mg organic material could be extracted (see also Table 2). After column liquid chromatographic separation of the crude material, 0.54mg of JC2 and and 0.74mg of JC3, respectively, remained. From each sample about 70% was used for ICPMS, about 10% for GC/MS, while only 20% was available for subsequent demetallation in order to analyse the respective free bases. Though these demetallation experiments were carried out very carefully, according to the procedure described in detail by Chicarelli (Chicarelli, M.I. 1985, Section IV), demetallation failed not only for the first vanadylporphyrin fraction JC3 but also for the nickelporphyrin fraction JC2. The small amounts of metalloporphyrins in both samples were obviously quantitatively decomposed during the treatment with methanesulfonic acid.
The unknown degradation products were not further investigated.

The demetallation of the quantitatively more important fraction JC5 was more successful. The respective AFID chromatogram is shown in Fig.81.

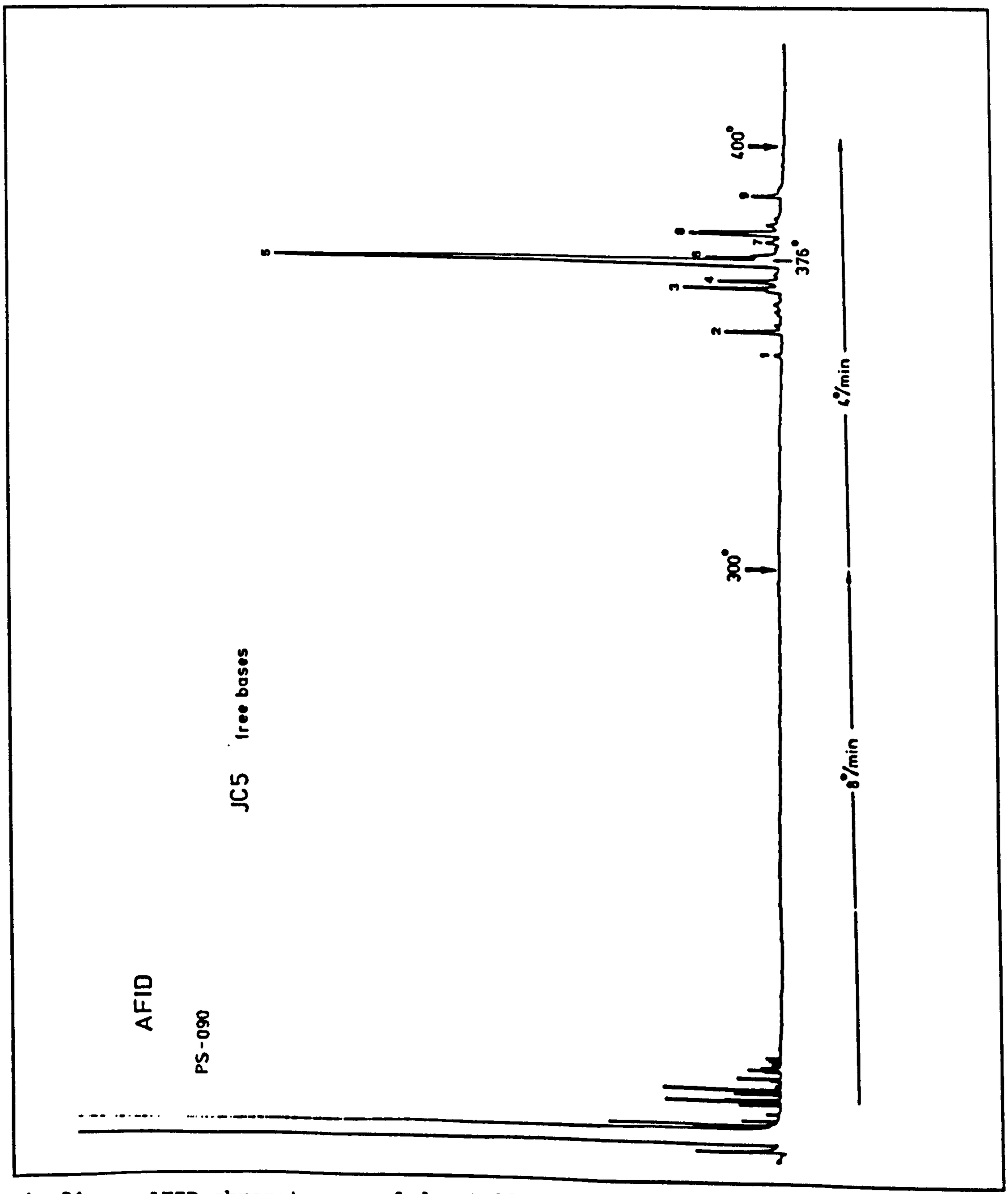


Fig.81 AFID chromatogram of demetallated JC5.

The transfer to GC/MS leads to the loss of several trace components, as already discussed in a corresponding context with the metalloporphyrins.

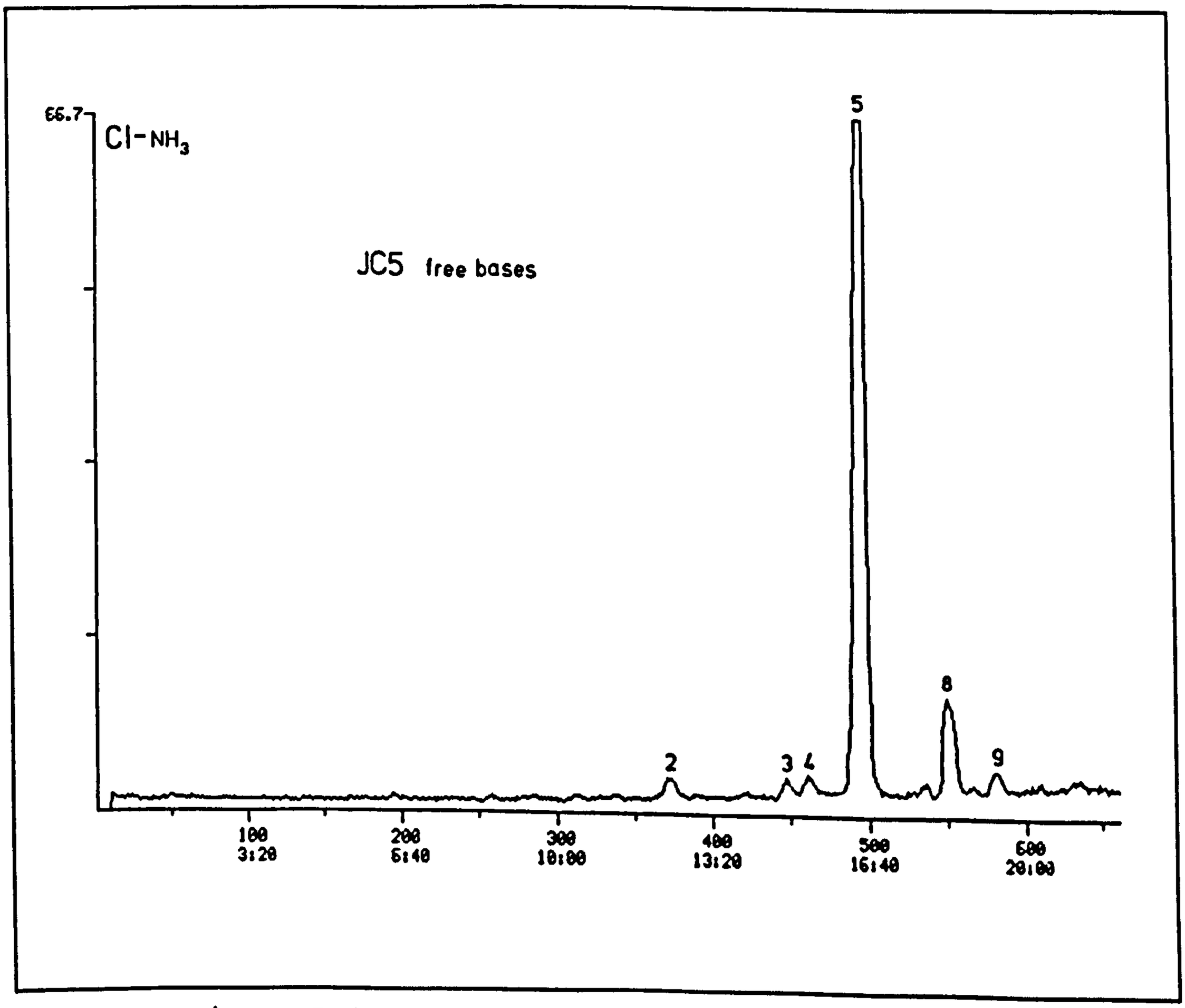


Fig.82 CI/NH_3-TIC chromatogram of demetallated fraction JC5.

However, the distribution obtained for the metalloporphyrins of JC5 (Fig.72 and Table 5) is confirmed by the analysis of the respective free bases as shown in the graphic presentation below.

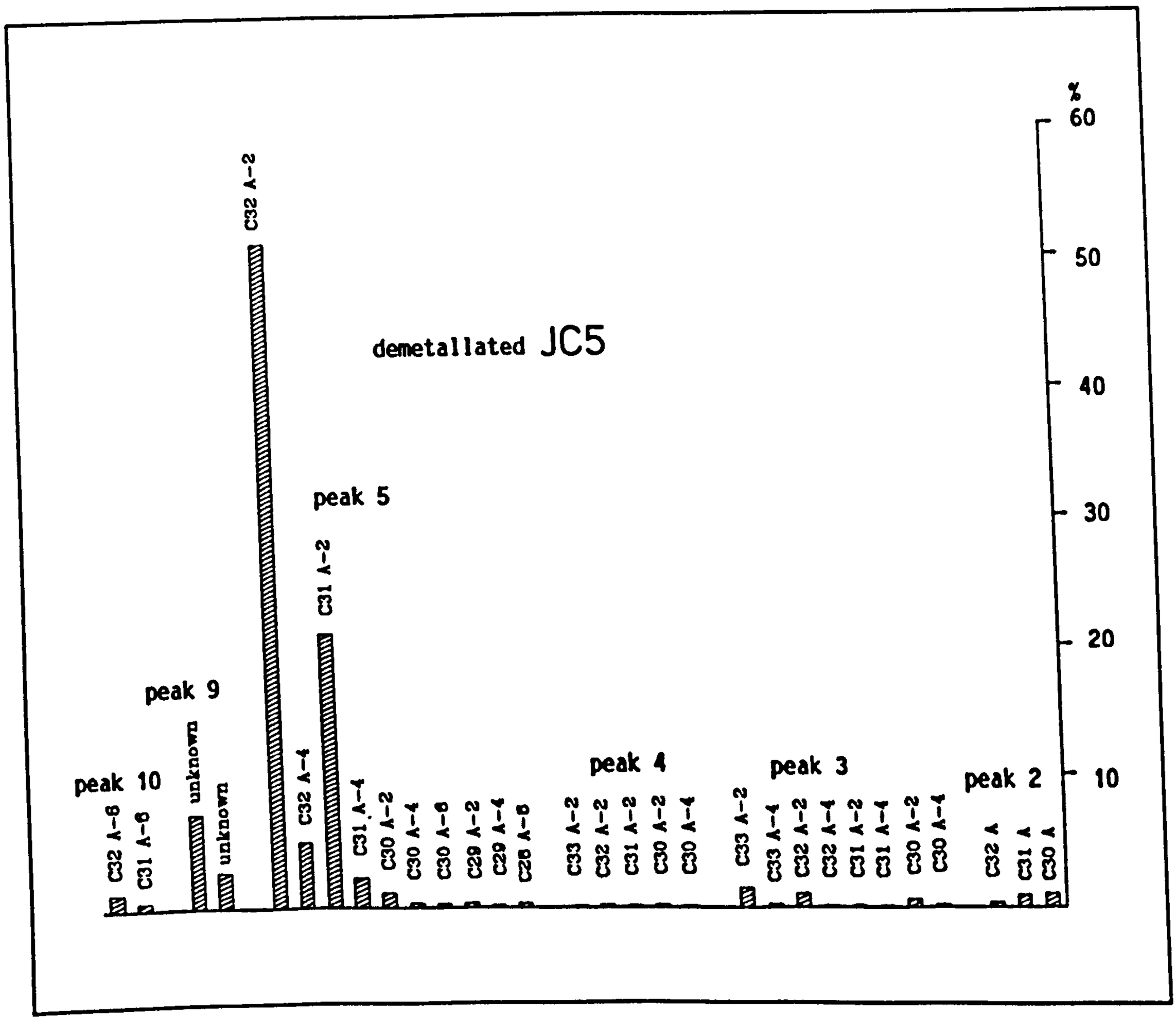


Fig.83 Graphical presentation of the free base porphyrin distribution of JC5.

Two main components of JC5, characterised as C_{32} A-2 and C_{31} A-2 porphyrins, covered by GC peak 5, were chosen in order to demonstrate the scope and also the limitation of GC/CIMS data in the structure analysis of porphyrins in naturally occurring mixtures.

V.2.2.1. GC/CIMS Investigation of Three C_{32} A-2 Reference Compounds

For this purpose three C_{32} A-2 porphyrins, structurally defined by NMR, were available as references, and analysed under identical conditions as was demetallated JC5.

The CI/NH_3 mass spectra of these three reference components 3,8-diethyl-2,7,12,18-tetramethyl-15,17(15^1-methyl)propanoporphyrin, 15,17-butano-3,8,-diethyl-2,7,12,18-tetramethylporphyrin and 13, 15-ethano-3,8,17-triethyl-2,7,12,18-tetramethylporphyrin are depicted in Figs.84-86.

Porphyrins are generally classified according the number of differently substituted pyrrole rings (A_4, A_3B, ABC_2 or ABCD) which they may contain. In the following fragmentation schemes 2-4, the designations A,B and C distinguish only the presence of different pairs of substituents on pyrrole rings but do not further distinguish positional isomers of the same two substituents on a particular pyrrole ring. Moreover, the individual fragmentation schemes are separated in a chemical ionisation (CI) pathway (pathway II, Section IV.2.12.1) termed 2a-4a, and a charge exchange (CE) pathway (pathway I, Section IV.2.1.2.1) termed 2b- 4b.

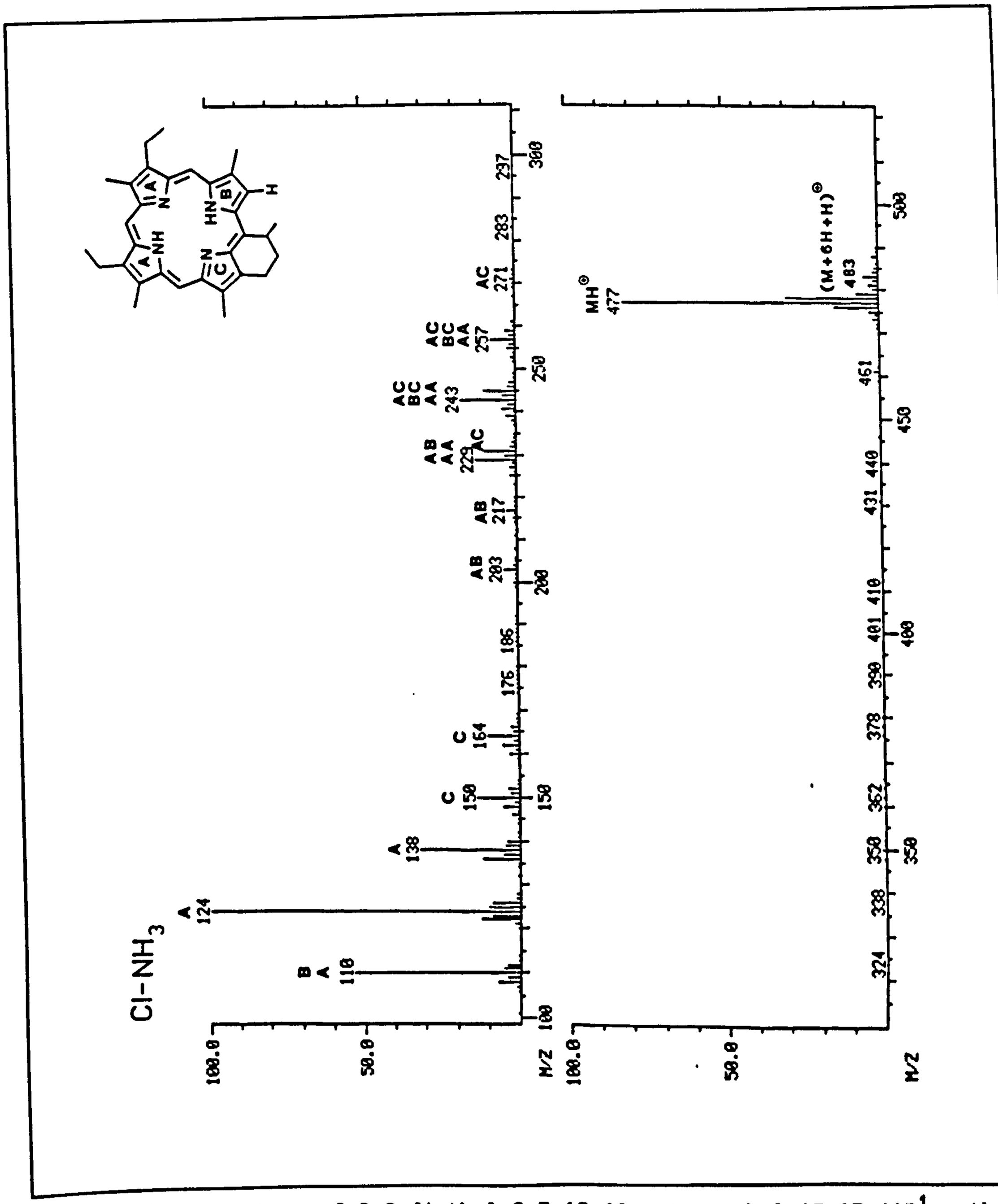
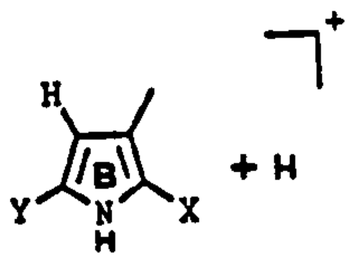
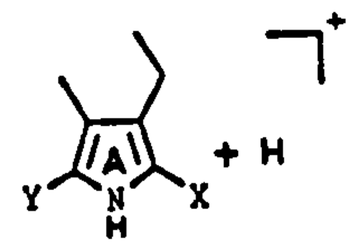


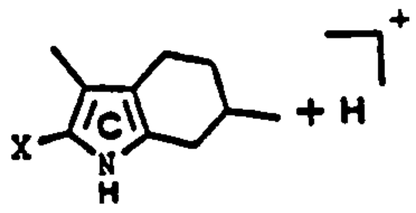
Fig.84 CI/NH_3 spectrum of 3,8-diethyl-2,7,12,18-tetramethyl-15,17-(15^1-methyl) propanoporphyrin.



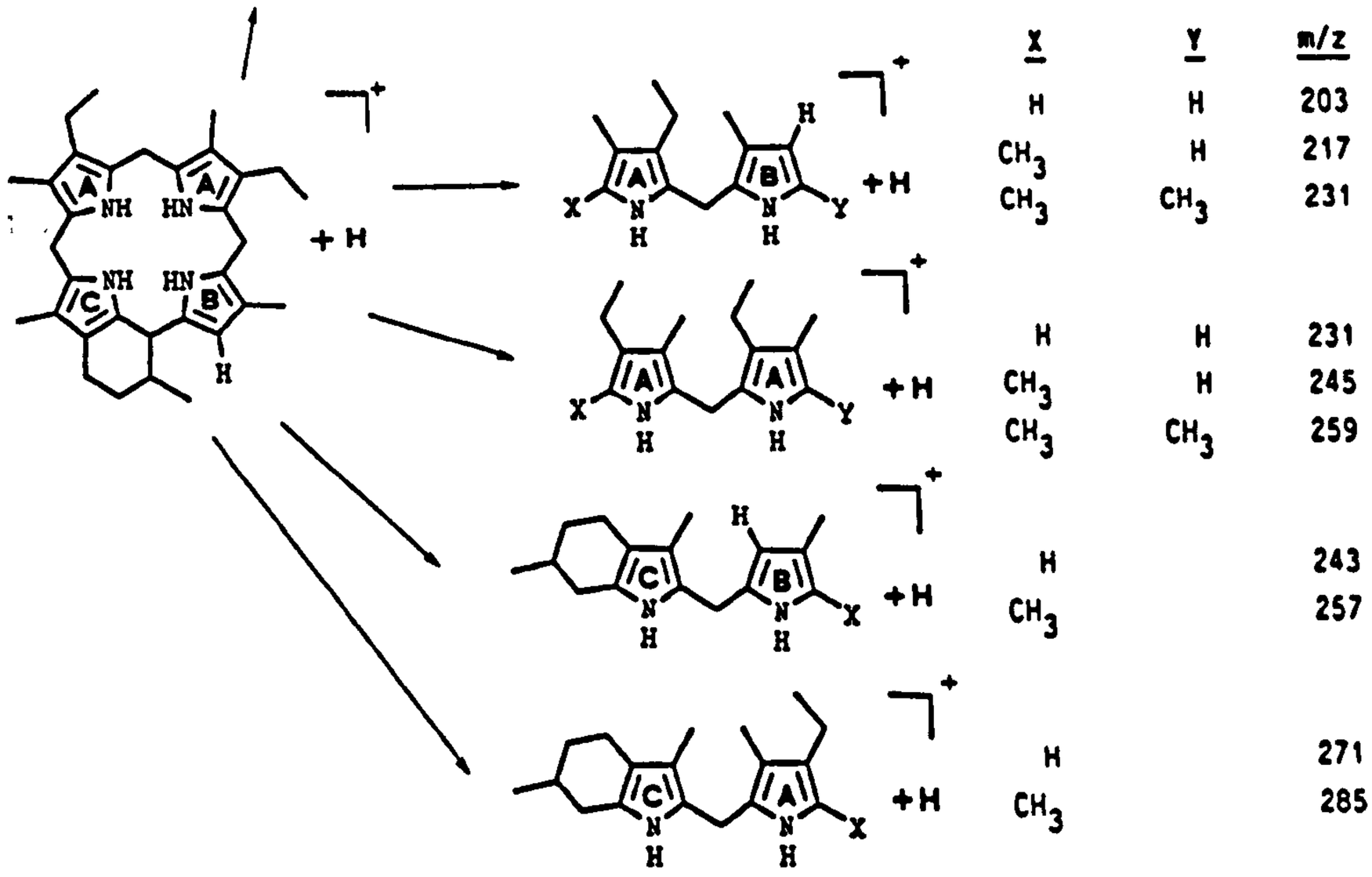
X	Y	m/z
H	H	82
CH_3	H	96
CH_3	CH_3	110



X	Y	m/z
H	H	110
CH_3	H	124
CH_3	CH_3	138

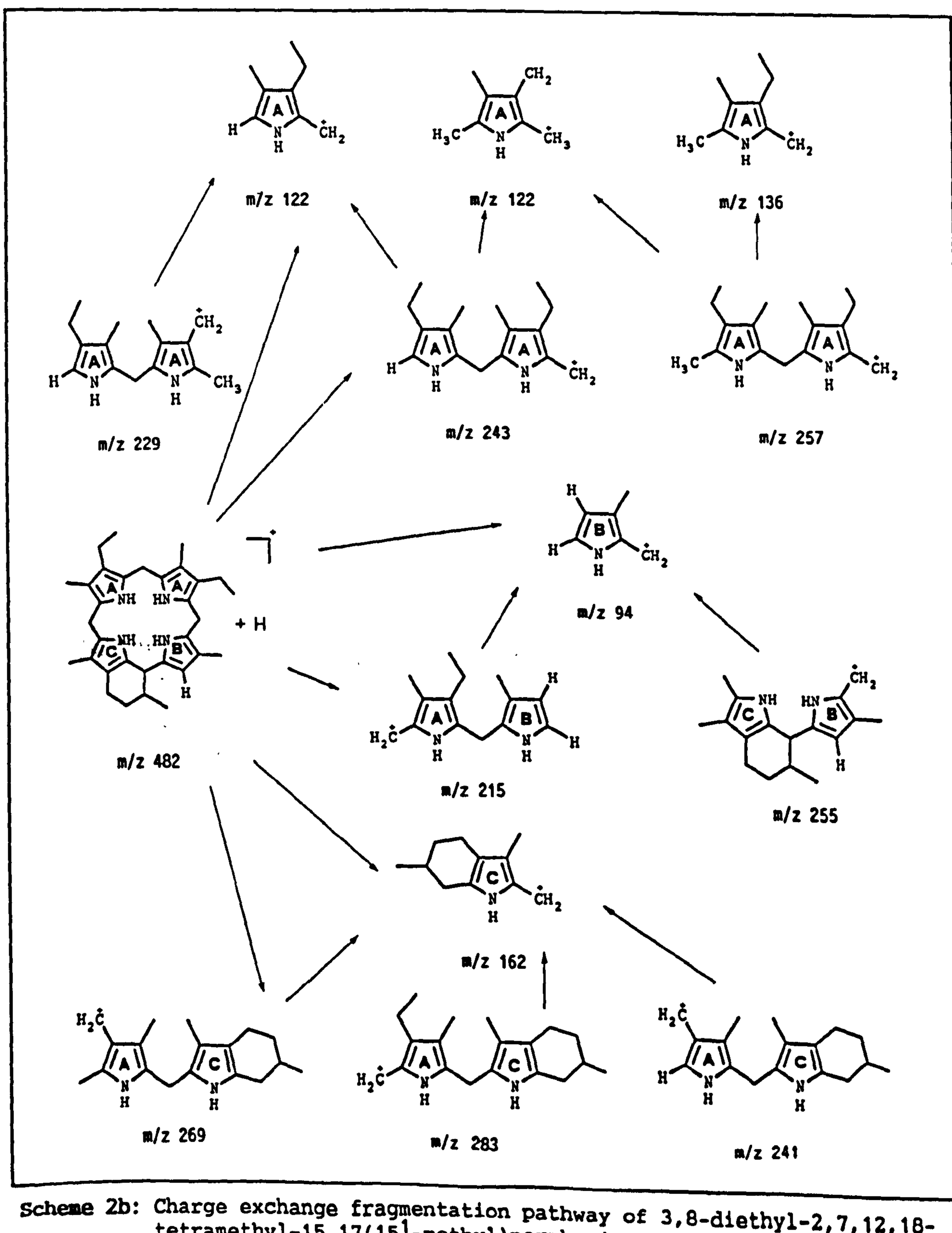


X	Y	m/z
H	H	150
CH_3	H	164



X	Y	m/z
H	H	203
CH_3	H	217
CH_3	CH_3	231
H	H	231
CH_3	H	245
CH_3	CH_3	259
H		243
CH_3		257
H		271
CH_3		285

Scheme 2a: CI/NH_3 fragmentation pathway of 3,8 -diethyl-2,7,12,18-tetramethyl-15,17(15^1-methyl)propanoporphyrin (C_{32} CAP me-6m.ring).



Scheme 2b: Charge exchange fragmentation pathway of 3,8-diethyl-2,7,12,18-tetramethyl-15,17(15^1-methyl)porphyrin.

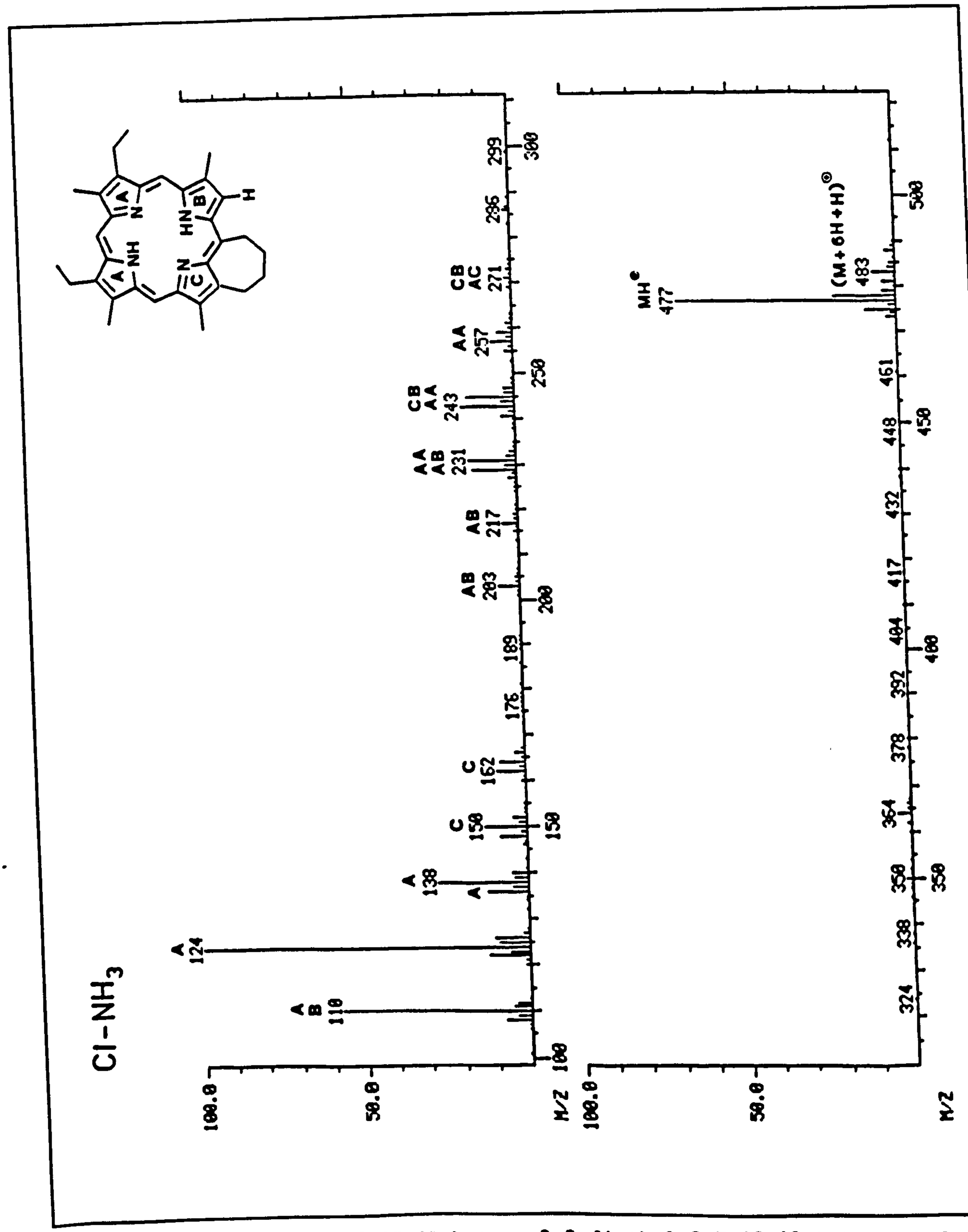
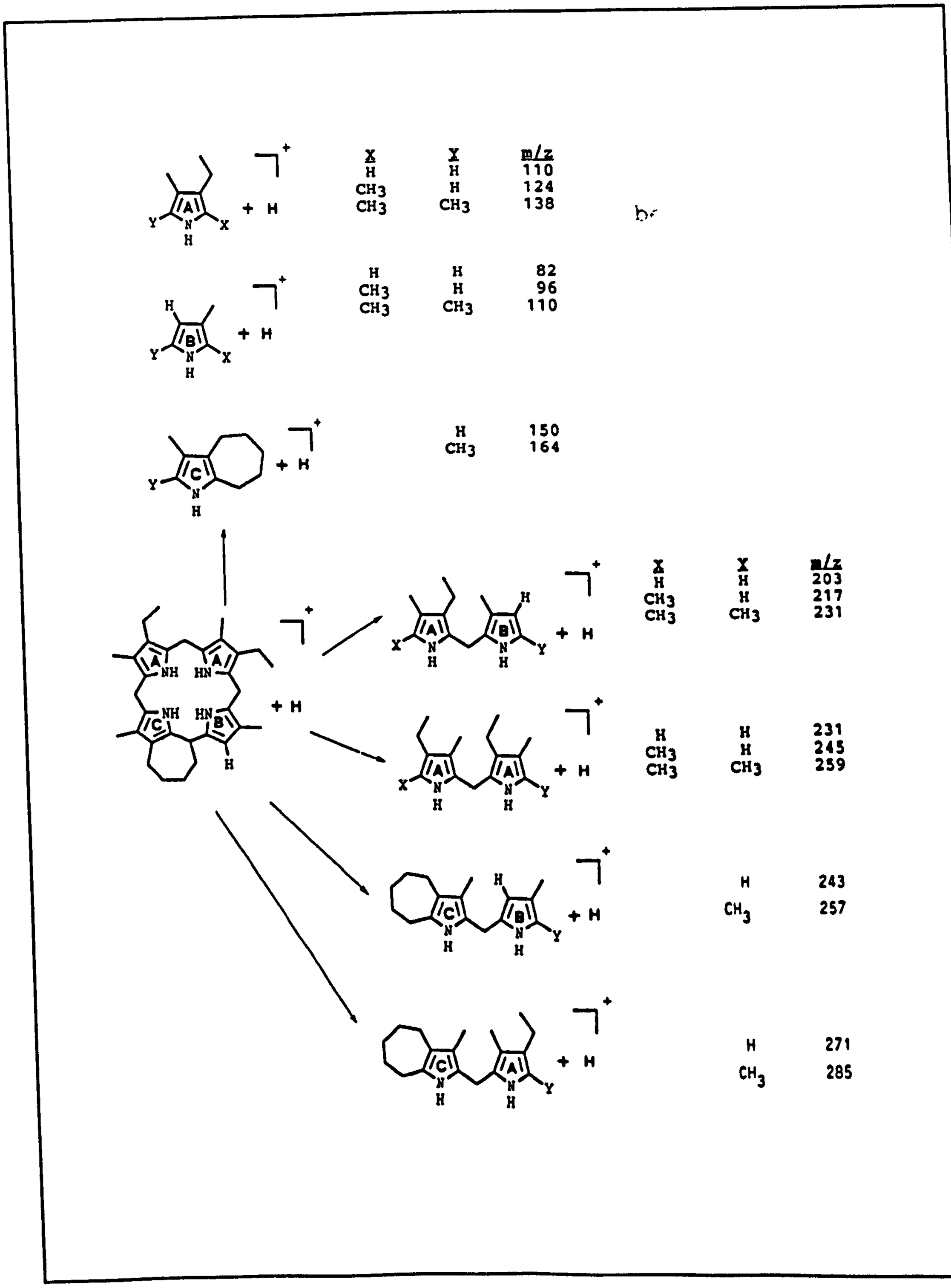
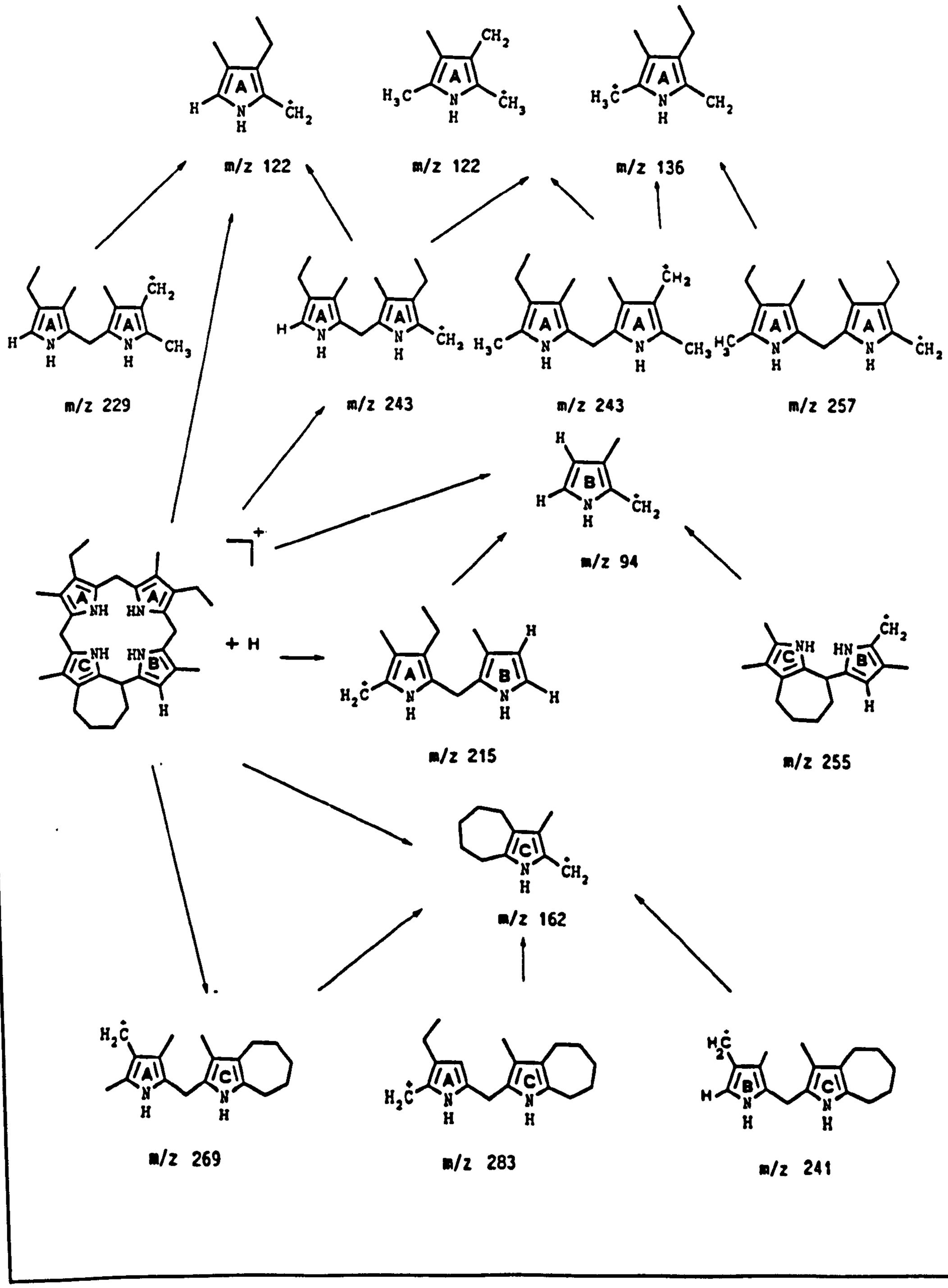


Fig.85 CI/NH_3 spectrum of 15,17-butano-3,8-diethyl-2,7,12,18-tetramethyl-porphyrin.



Scheme 3a: CI/NH_3 fragmentation pathway of 15,17-butano-3,8-diethyl-2,7,12,18-tetramethylporphyrin (C_{32} CAP 7-m.ring).



Scheme 3b: Charge exchange fragmentation pathway of 15,17-butano-3,8-diethyl-2,7,12,18-tetramethylporphyrin.

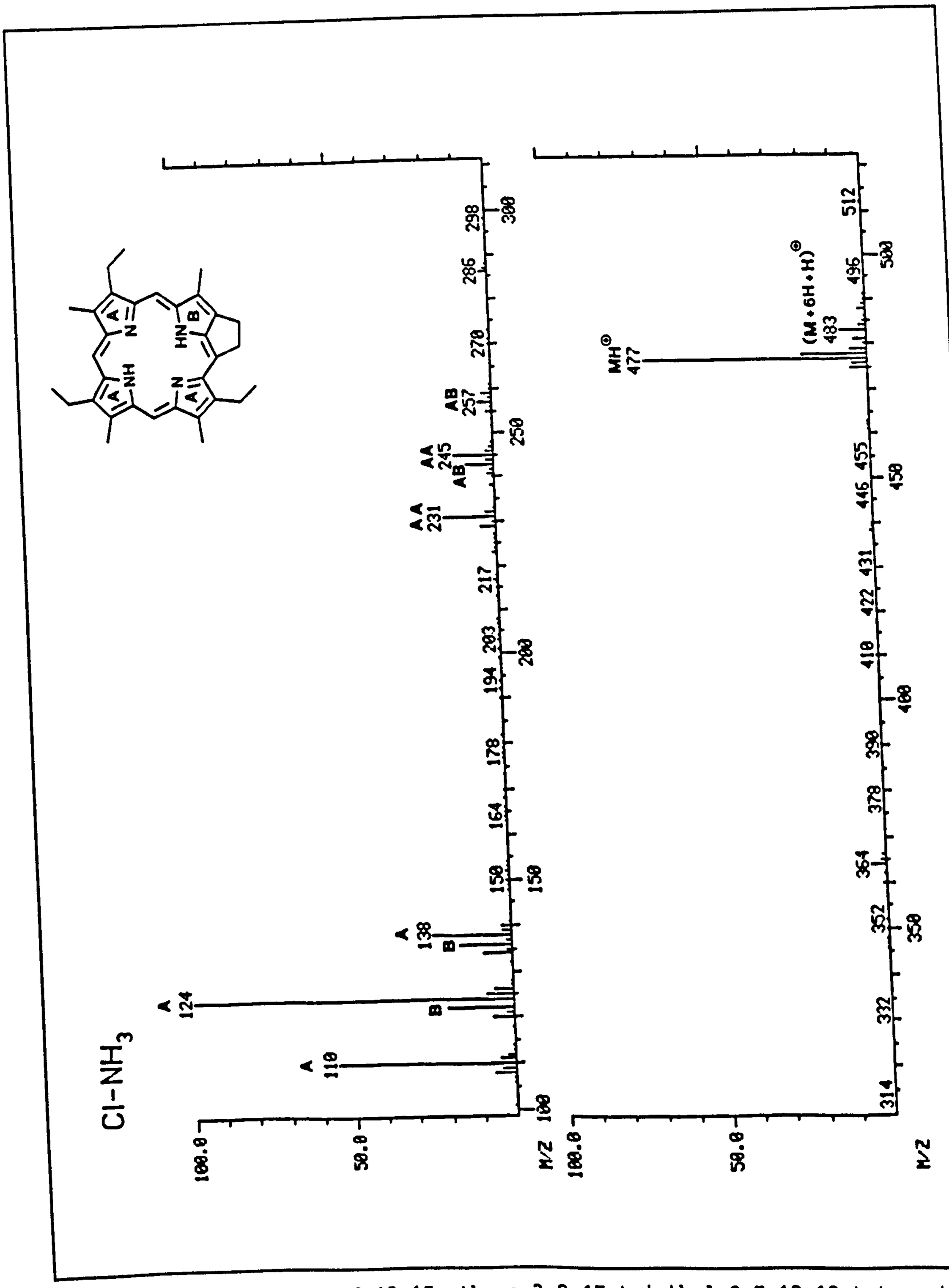
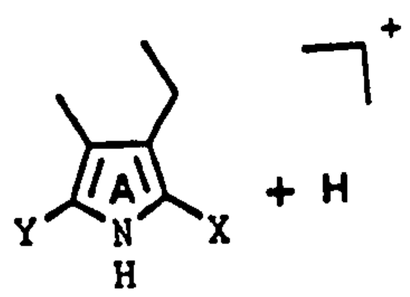
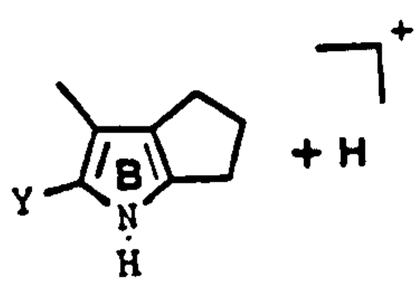


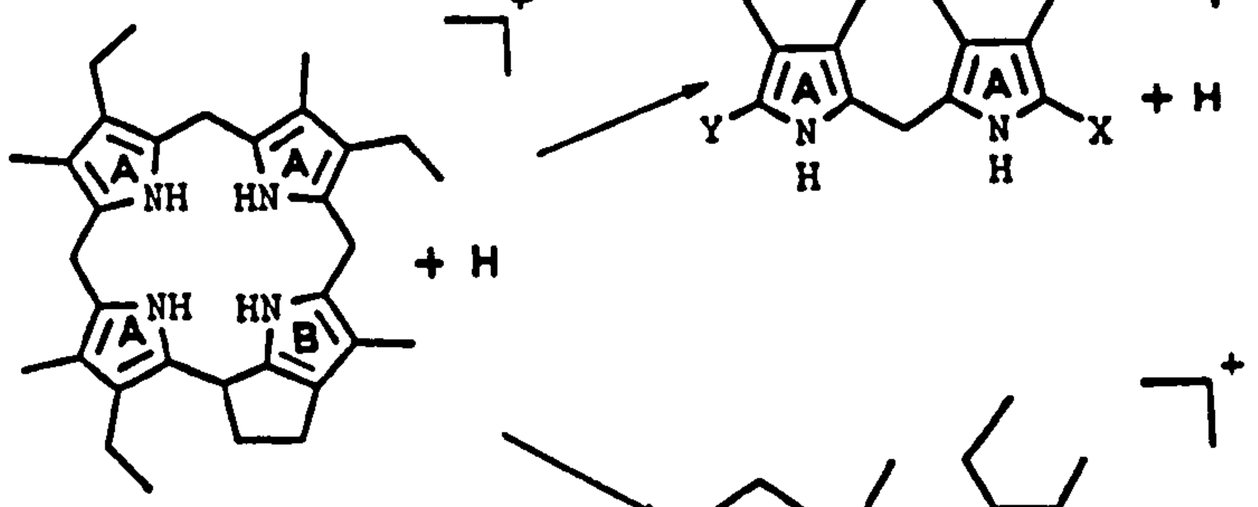
Fig.86 CI/NH_3 spectrum of 13,15-ethano-3,8,17-triethyl-2,7,12,18-tetramethyl-porphyrin.



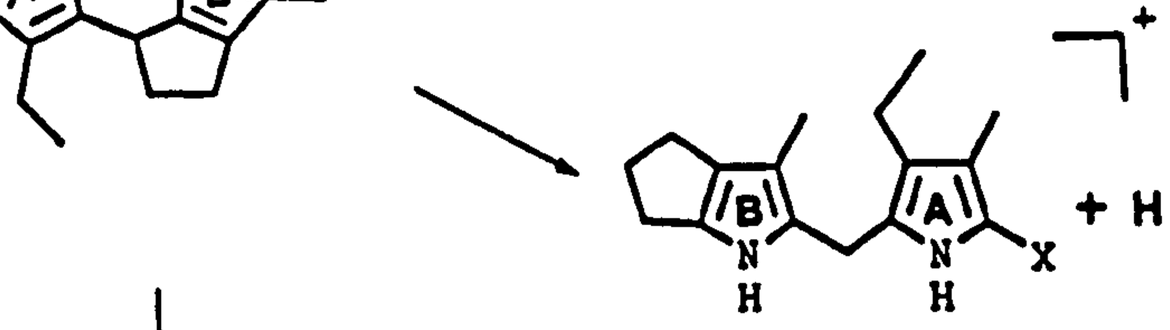
X	Y	m/z
H	H	110
CH_3	H	124
CH_3	CH_3	138



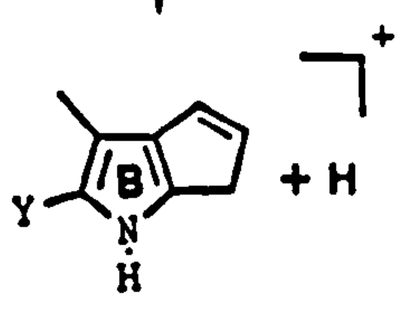
X	Y	m/z
H	H	122
CH_3	CH_3	136



X	Y	m/z
H	H	217
CH_3	H	231
CH_3	CH_3	245

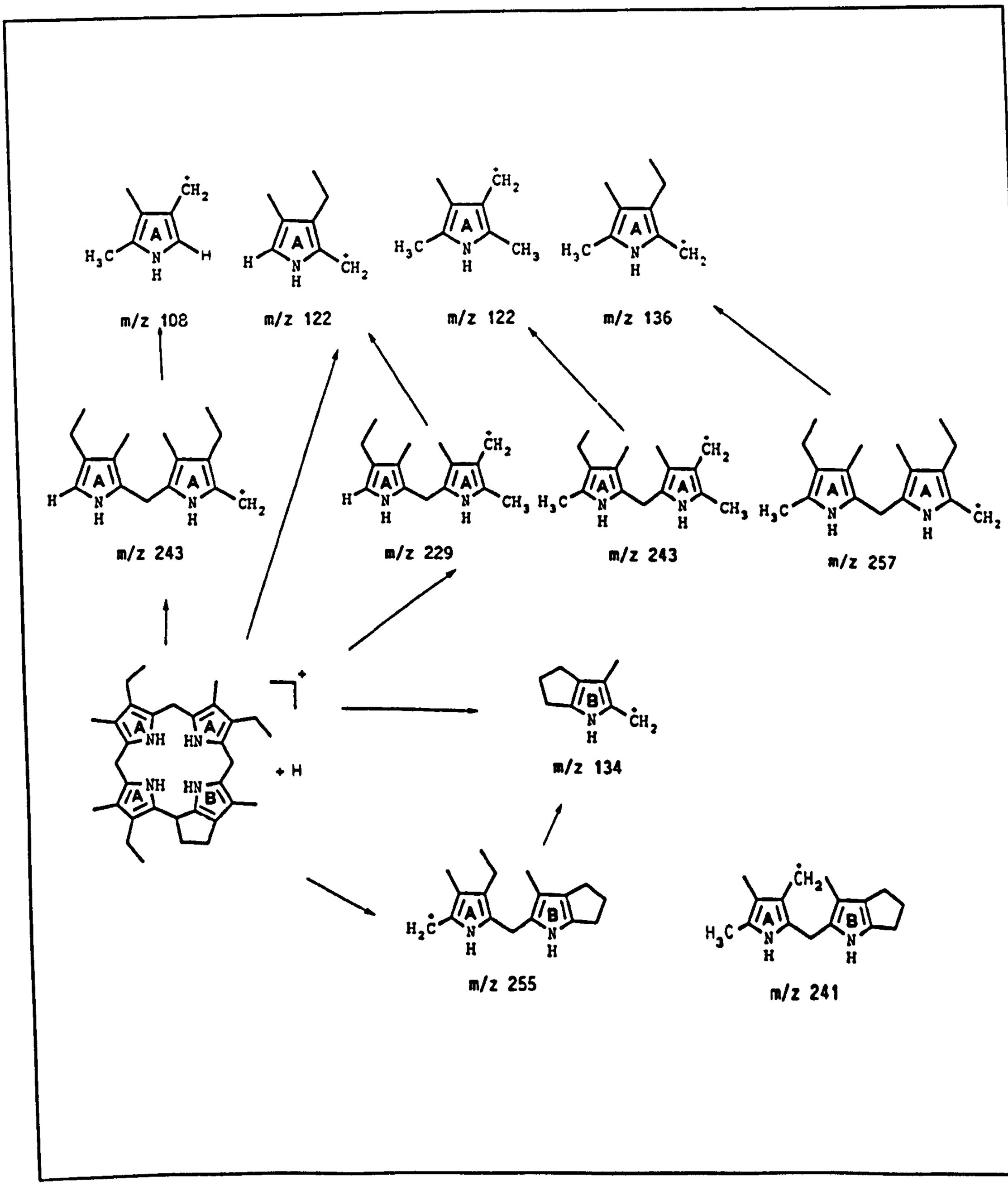


X	Y	m/z
H	H	243
CH_3	CH_3	257



Y	m/z
H	120
CH_3	134

Scheme 4a: CI/NH_3 fragmentation pathway of 13,15-ethano-3,8,17-triethyl-2,7, 12,18-tetramethylporphyrin (C_{32} DPEP).



Scheme 4b: Charge exchange fragmentation pathway of 13,15-ethano-3,8,17-triethyl-2,7,12,18-tetramethylporphyrin.

At a first glance, the spectra of the three reference porphyrins look similar. Initial attack by the reagent gas results in the formation of protonated quasi-molecular ions at m/z 477. Further hydrogenation leads to protonated porphyrinogens, m/z 483 $(M+6H+H)^+$, with rel. ion intensity of 10% for DPEP and only about 3% for C_{32} CAP me-6-m.ring.

But, even with NH_3 as a reagent gas, chemical ionisation is accompanied by charge exchange fragmentation. The amount of charge exchange ions appears to depend on the nature of the exocyclic ring system. Secondary ion formation, directly from the porphyrinogen molecular-ions or by benzylic cleavage of tripyrrolic- or dipyrrolic fragment ions, is more pronounced for the references bearing six- or seven membered exocyclic ring systems, presumably enhanced by the carrier gas (see Section IV.2.4.1).

Nevertheless, the partial spectra from m/z 100-200 and m/z 200-300, representing the mono- and dipyrrolic range, respectively, show some differences among the three isomers.

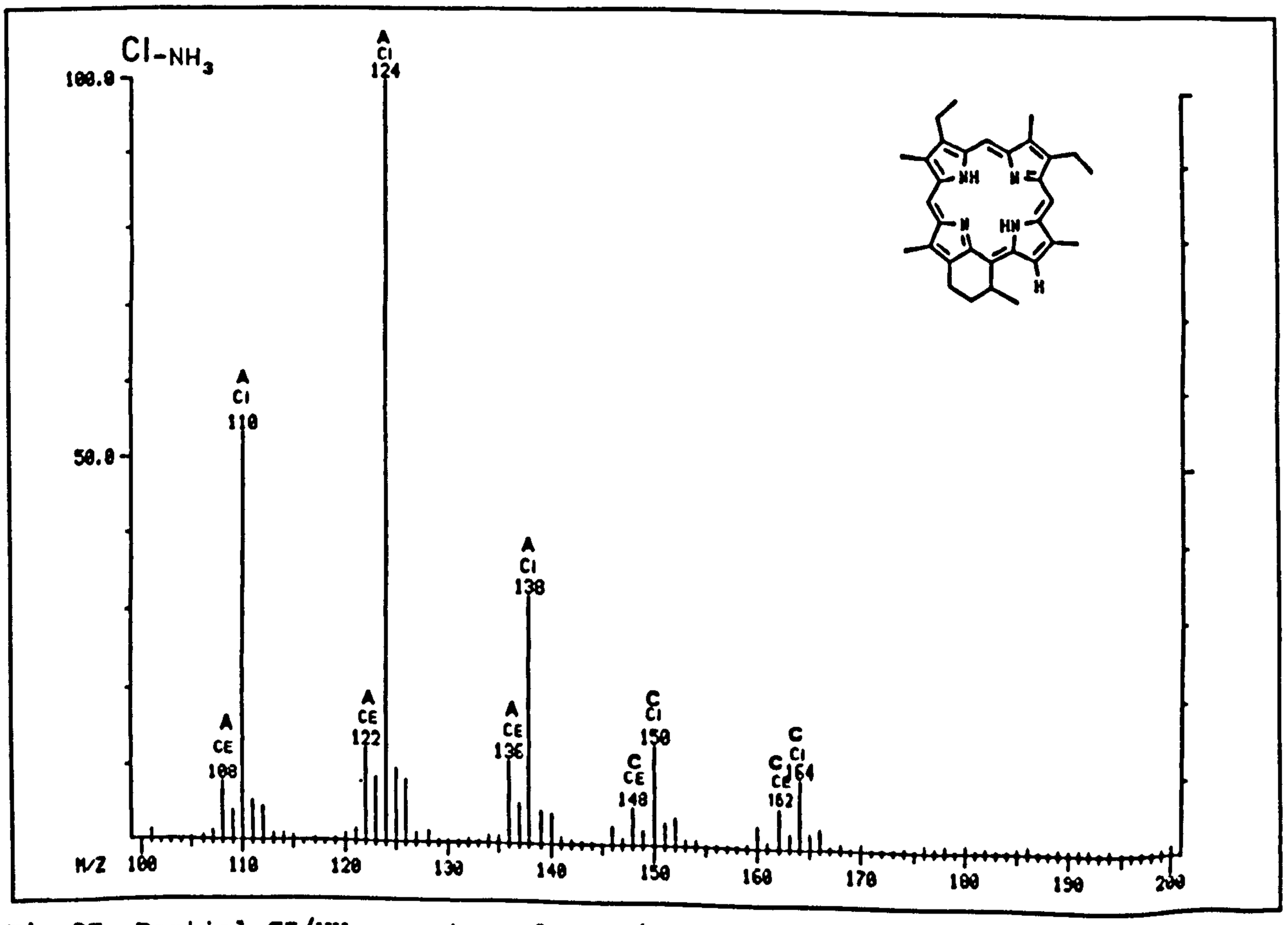


Fig.87 Partial CI/NH_3 spectrum from m/z 100-200 of 3,8-diethyl-2,7,12,18-tetramethyl-15,17(15^1-methyl)propanoporphyrin.

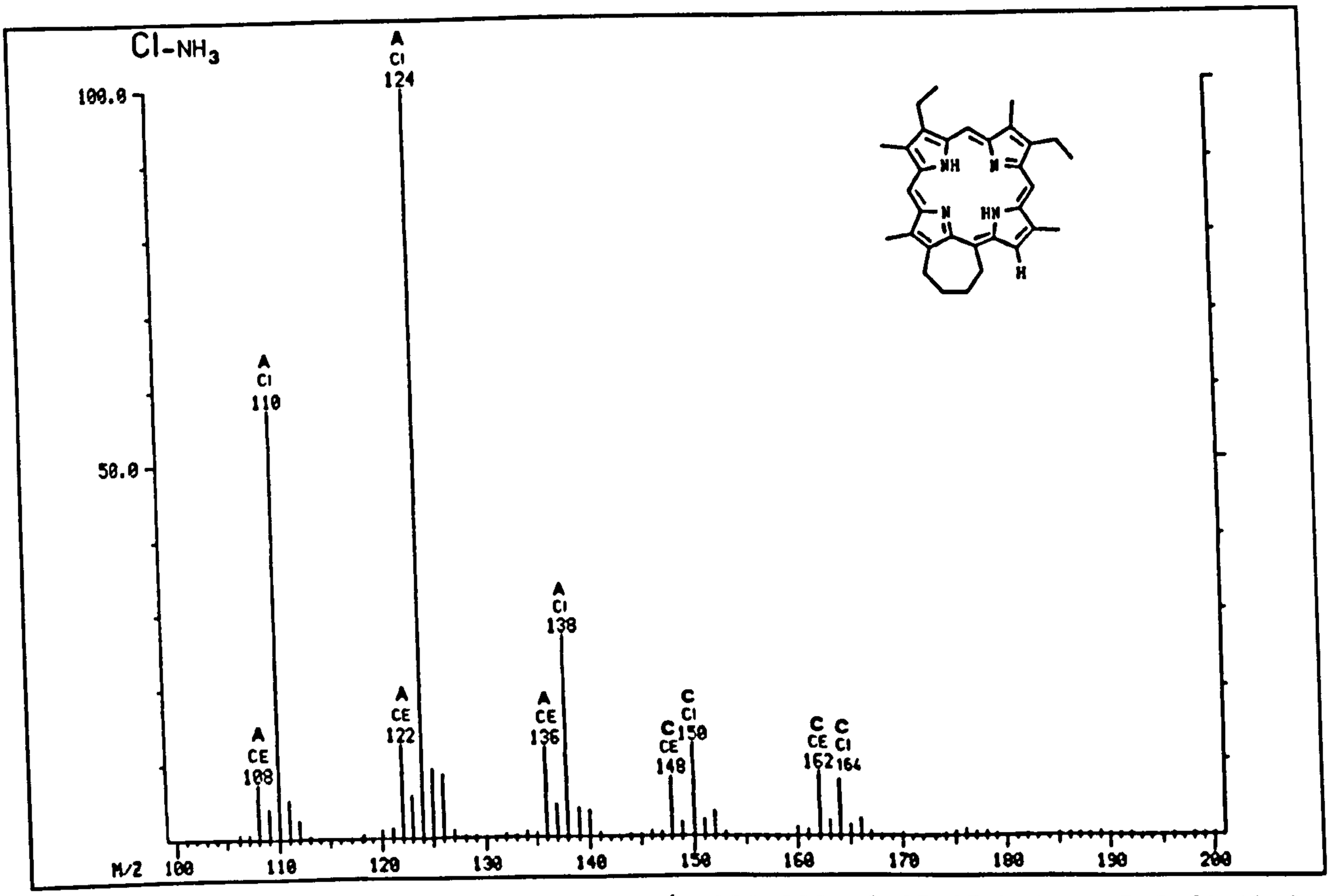


Fig.88 Partial CI/NH_3 spectrum from m/z 100-200 of 15,17-butano-3,8-diethyl-2,7,12,18-tetramethylporphyrin.

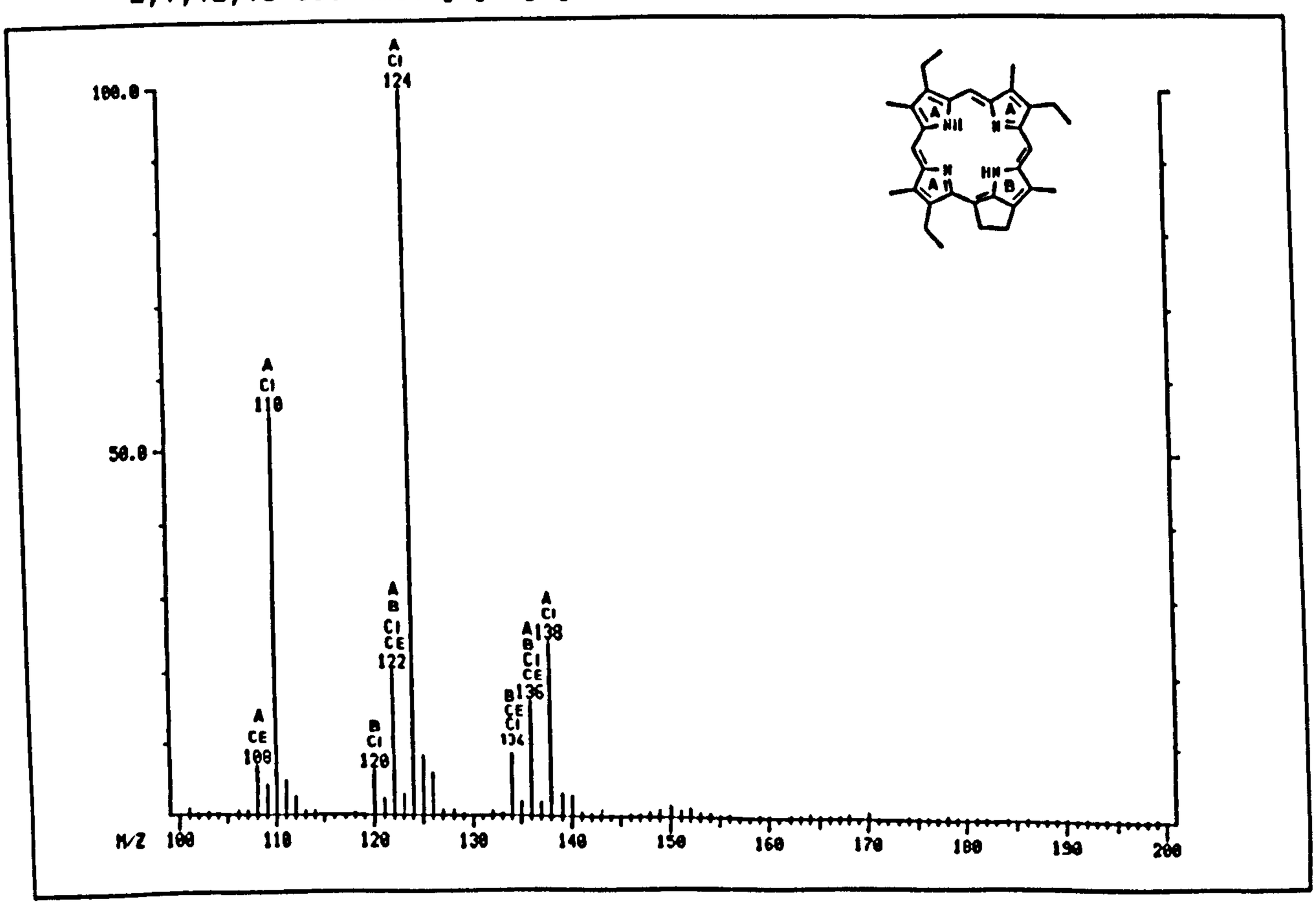


Fig.89 Partial CI/NH_3 spectrum from m/z 100-200 of 13,15-ethano-3,8,17-triethyl-2,7,12,18-tetramethylporphyrin.

The monopyrrolic region exhibits intensive non specific protonated fragments at m/z 110, 124 and 138, and reduced but structurally significant signals at m/z 150 and 164, representative for the (C)pyrrole segments in both C_{32} Cap 7-m.ring an C_{32} CAP 6-me- 6m.ring.

The dipyrrolic range exhibits four sets of doublets of protonated- and radical ions. The occurrence of the protonated species at m/z 231 and m/z 245, present in all three spectra, is highest for C_{32} DPEP and less intensive in the spectra of the other reference components.

Structurally significant tripyrrolic fragments are only of negligible abundance and do not provide additional information.

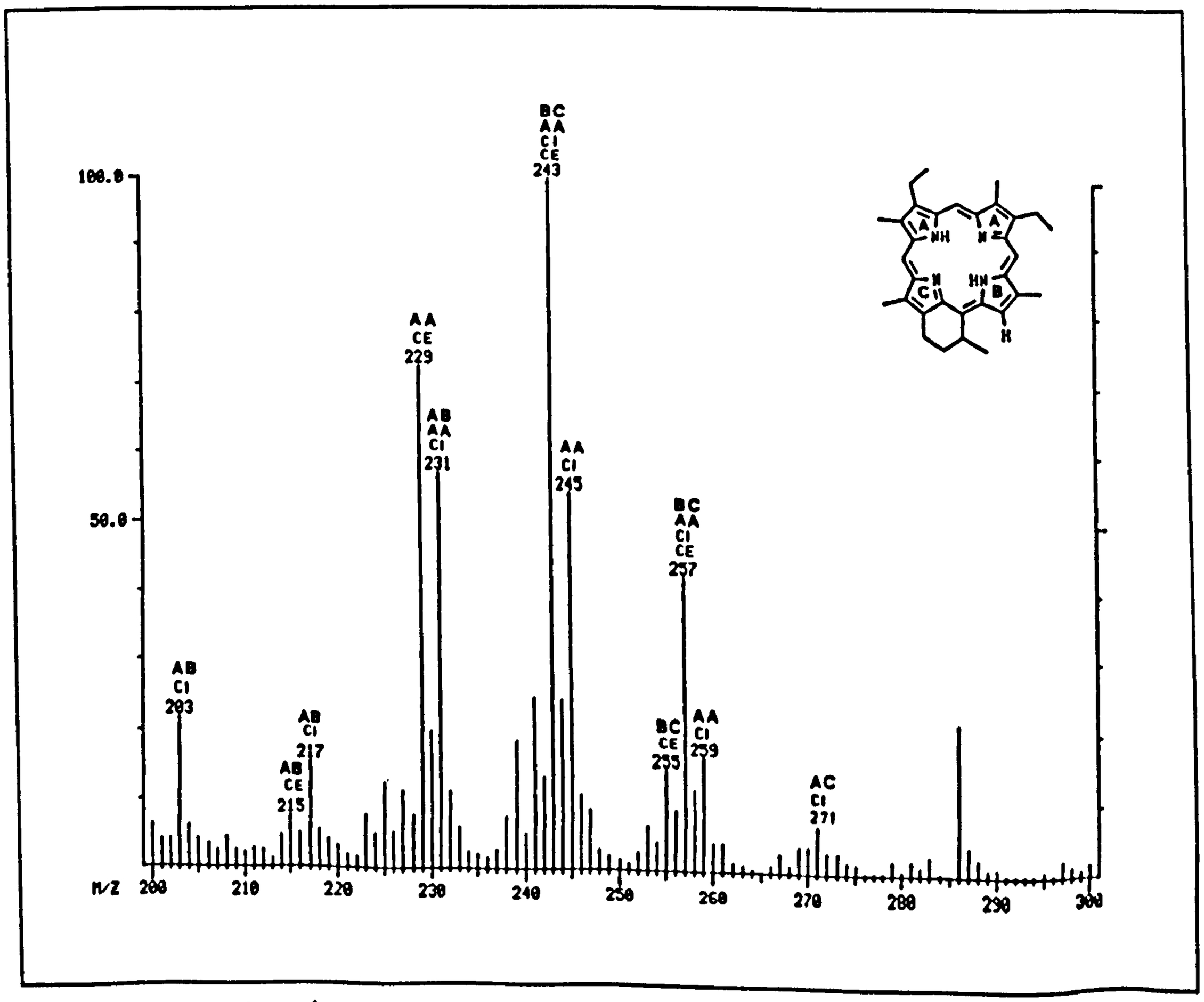


Fig.90 Partial CI/NH_3 spectrum from m/z 200-300 of 3,8,diethyl-2,7,12,18-tetramethyl-15,17(15^1-methyl)propanoporphyrin.

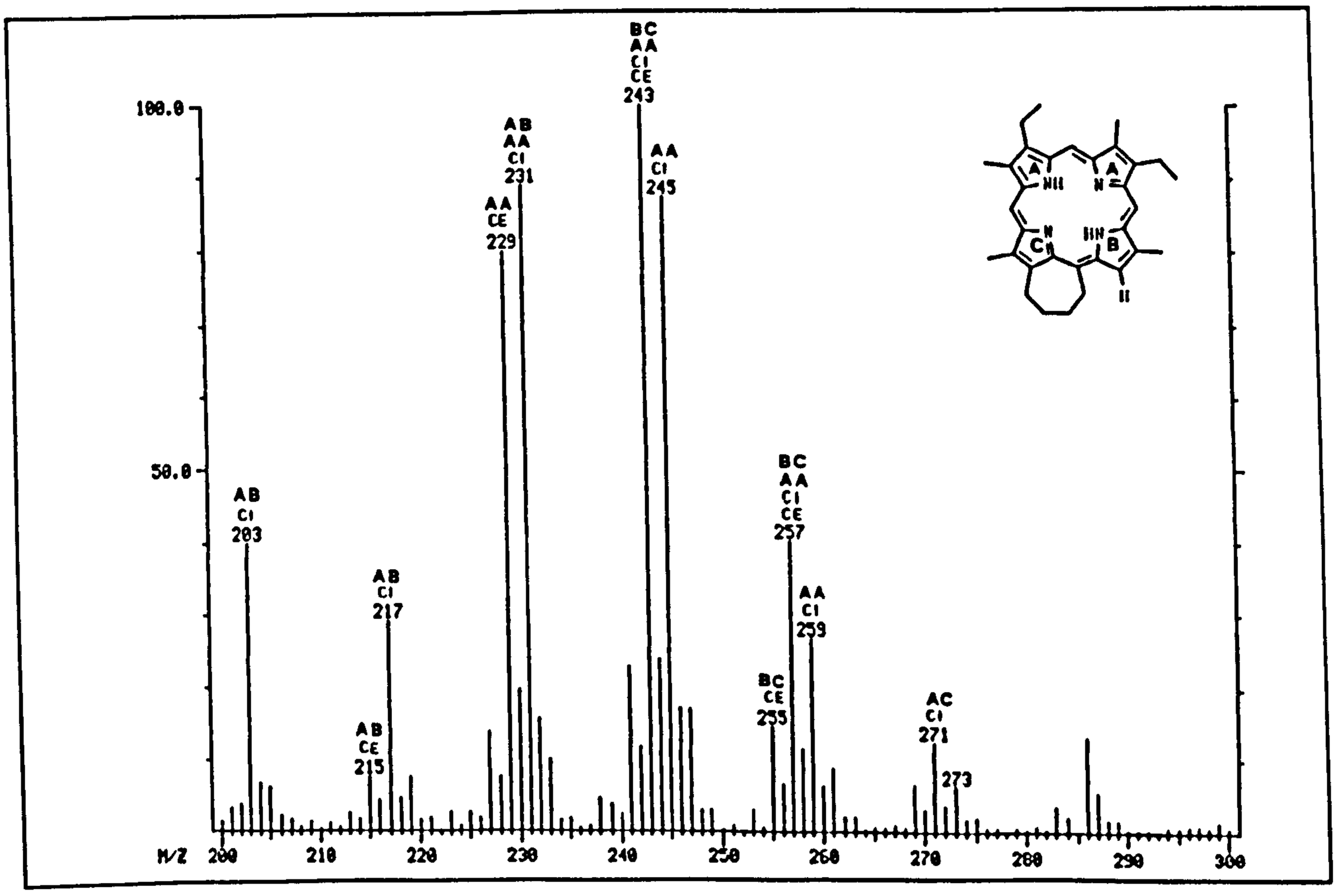


Fig.91 Partial CI/NH_3 spectrum from m/z 200-300 of 15,17-butano-3,8-diethyl-2,7,12,18-tetramethylporphyrin.

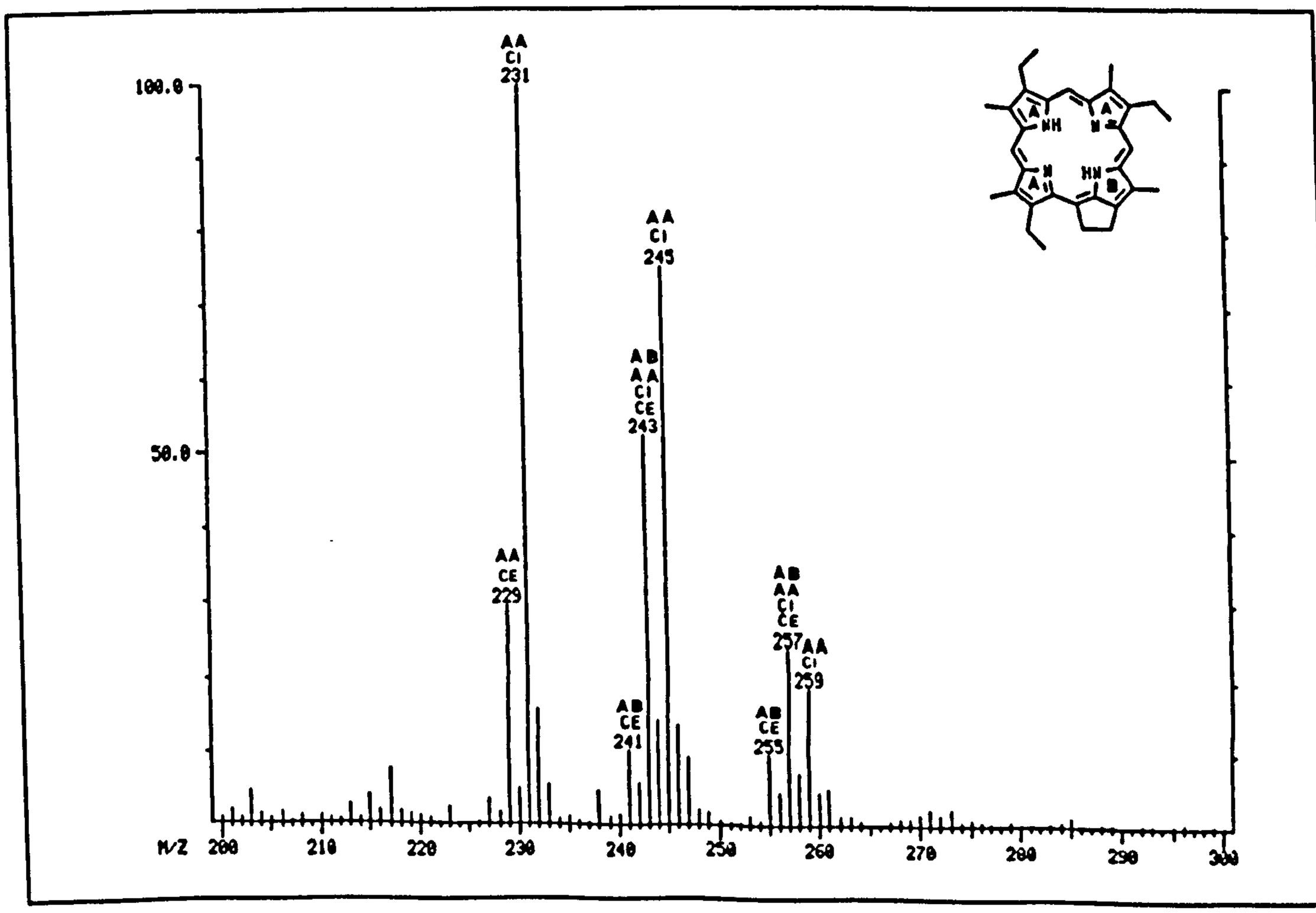


Fig.92 Partial CI/NH_3 spectrum from m/z 200-300 of 13,15-ethano-3,8,17-triethyl-2,7,12,18-tetramethylporphyrin.

When faced with the problem of using GC/CIMS data in order to elucidate the structures of unknown petroporphyrins, one has seriously to take into account the secondary reactions in the ion source. This point has already been mentioned in Section IV.2.4.. The mass selected chromatograms of one of the reference components reveal again, that in CI-spectra of porphyrins, the quasi-molecular ions and the respective fragment ions do not elute at the same time, and have therefore not the same peak maxima. Moreover, the fragment ions exhibit distinct tailing, overlapping with subsequent eluting peaks.

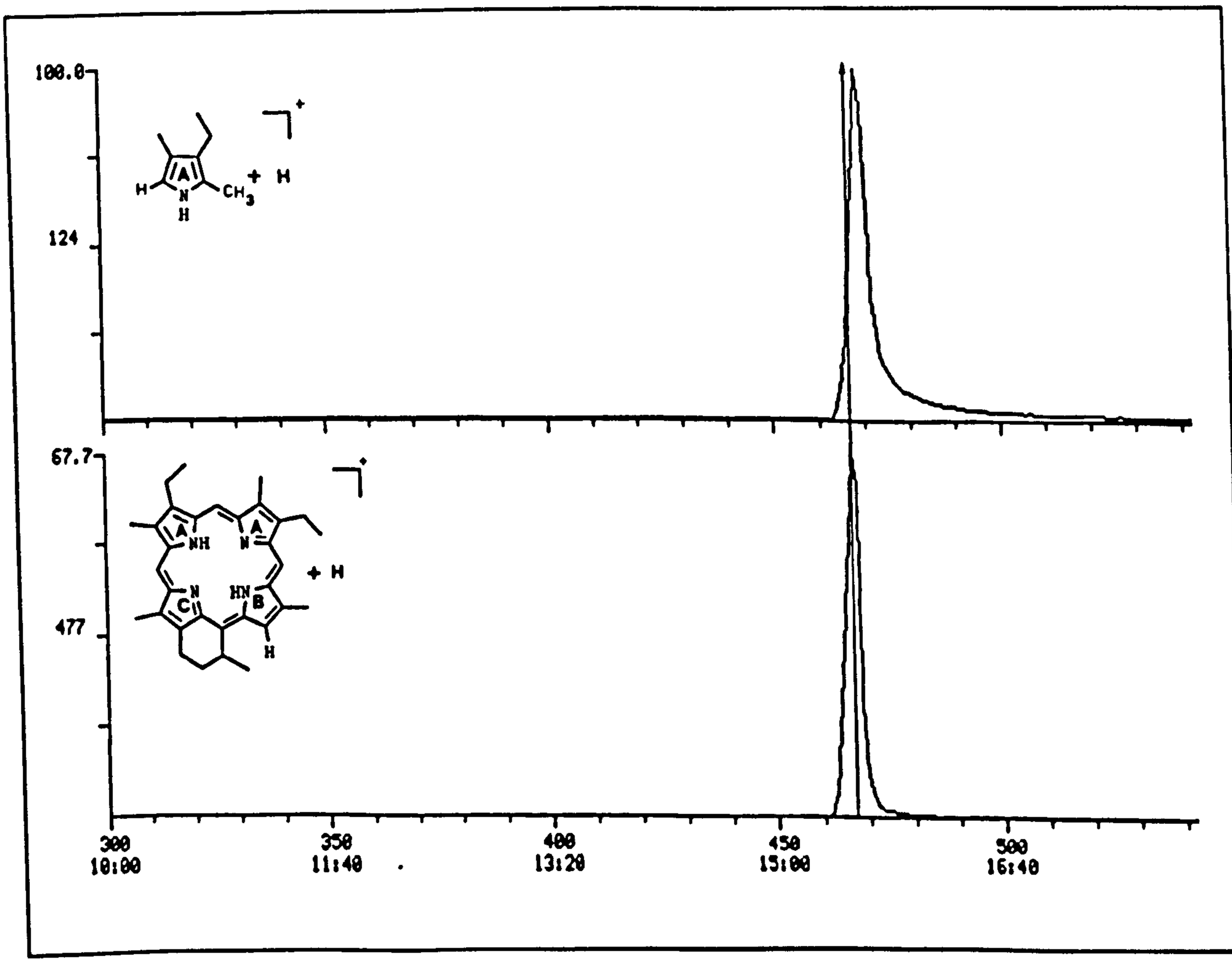


Fig.93 Selected CI/NH_3 ion current chromatogram of m/z 124 and m/477 of 3,8-diethyl-2,7,12,18-tetramethyl-15,17(15^1-methyl)propanoporphyrin.

In order to create reproducible data, summed spectra were taken at the maximum of the quasi-molecular ion (m/z 477) in the ion selected chromatogram.

V.2.2.2 Structure Assignment of Peak 5.

Bearing in mind the fragmentation pattern of the references components, the summed spectra taken at the front side of GC peak 5 (top of m/z 477) of demetallated JC5 compare well with that of reference C_{32} DPEP depicted in Fig.86.

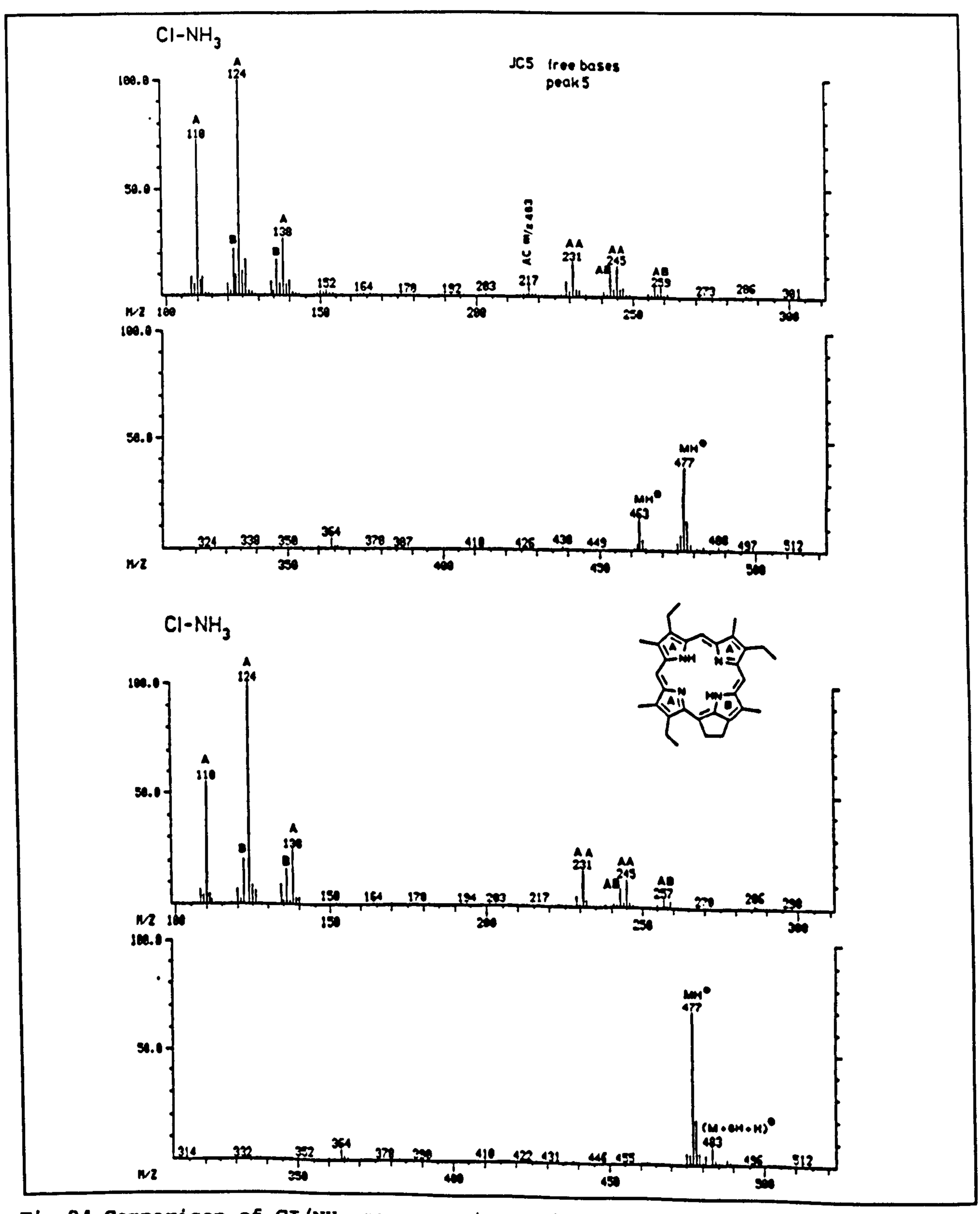


Fig.94 Comparison of CI/NH_3 mass spectrum of 13,15-ethano-3,8,17-triethyl-2,7, 12,18-teramethylporphyrin with peak 5 of demetallated JC5 (

The consequent interpretation, that the main component in the fraction JC5 is 13,15-ethano-3,8,17-triethyl-2,7,12,18-tetramethylporphyrin is further supported by GC retention data.
The AFID chromatograms in Figs.95 and 96 demonstrate that the three isomeric C_{32} A-2 porphyrins can be base line separated by gas chromatography and that the retention temperature of the main component in JC5 coincides with that of C_{32} DPEP.

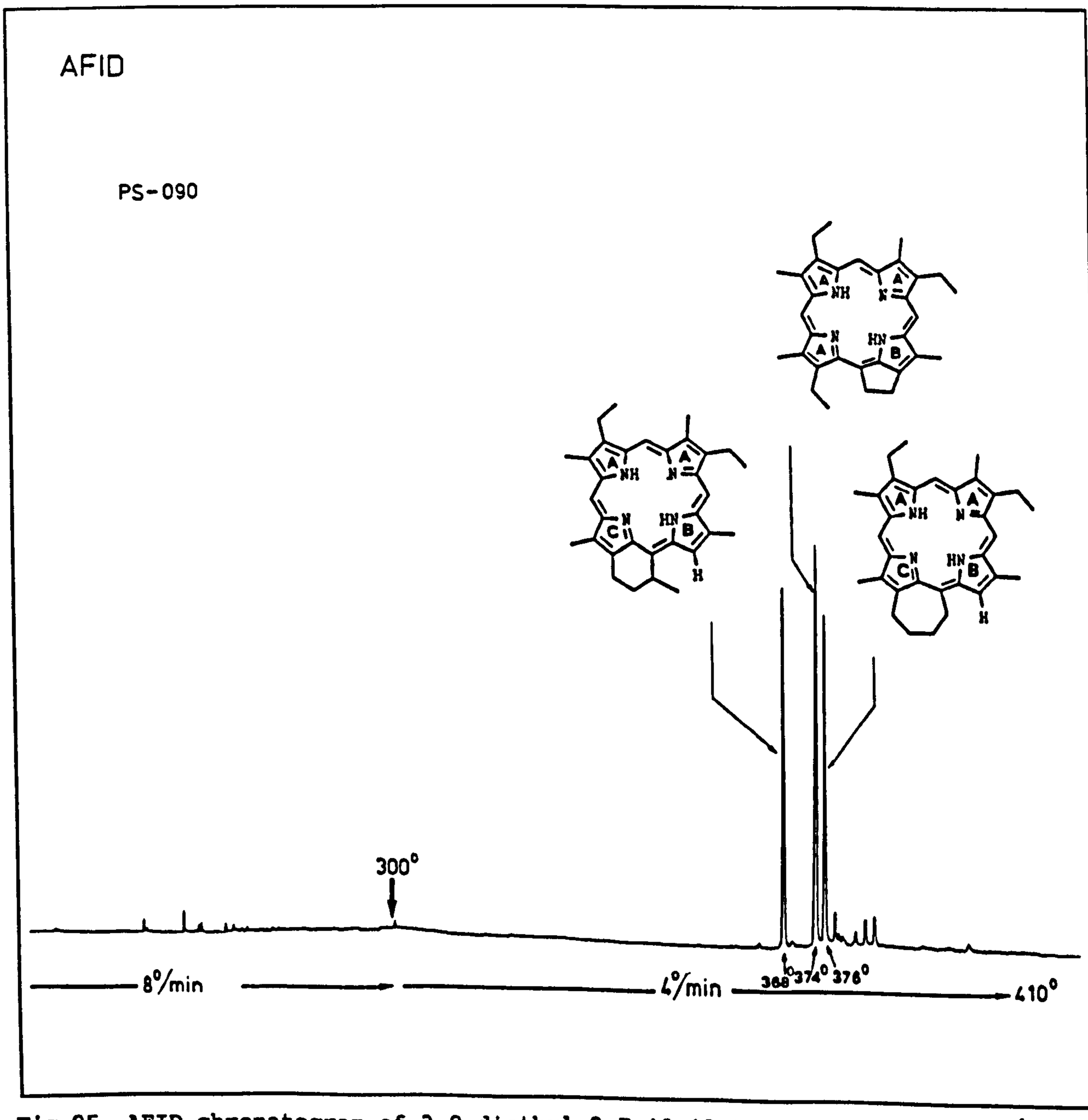


Fig.95 AFID chromatogram of 3,8-diethyl-2,7,12,18-tetramethyl-15,17(15^1-methyl)propanoporphyrin,15,17-butano-3,8-diethyl-2,7,12,18-tetramethyl-porphyrin and 13,15-ethano-3,8,17-triethyl-2,7,12,18-tetramethylporphyrin.

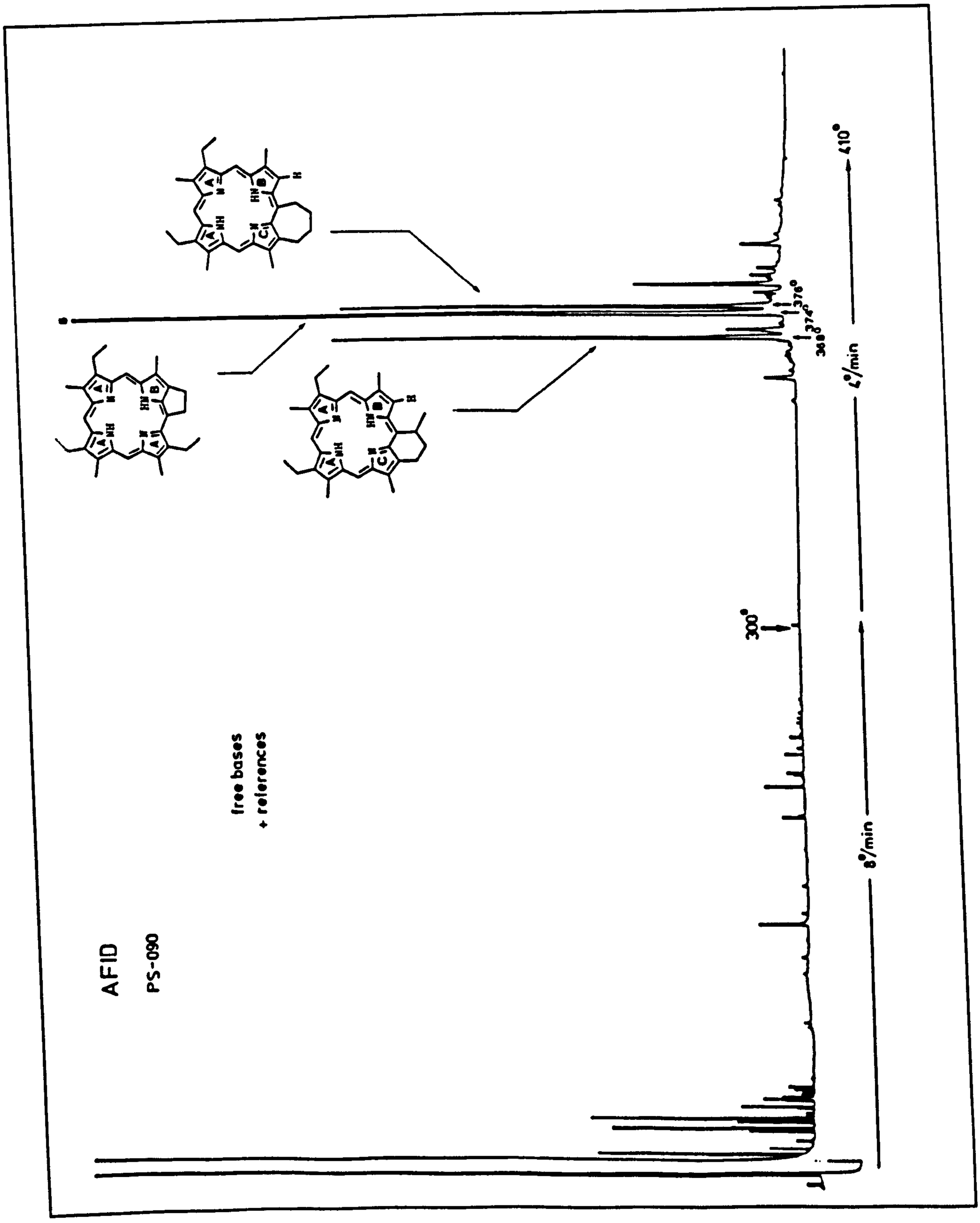


Fig.96 Co-injection of three C_{32} A-2 references and demetallated JC5.

This finding is in accordance with NMR investigations of Julia Creek oil shale reported by Fookes (Fookes, C.J.R. 1983).

GC peak 5 covers, beside the already identified C_{32} DPEP, a second main component indicated by a quasi-molecular ion at m/z 463. Since the fragmentation pattern, not only of the mono- but also of the dipyrrolic ions of this component correspond to the spectrum of C_{32} DPEP, it can be assigned to a homologue C_{31} DPEP, although covered by fragments of the foregoing component. The AC-segment of C_{31} DPEP is indicated by a protonated fragment at m/z 217 (see Fig.94). This example demonstrates again the fundamental difficulties in correlating fragment ions of non- or partially separated porphyrins with proper molecular ions, particularly on the backside of dominant GC peaks.

V.2.2.3. Assignment of "Unknown" Peak 8, in demetallated JC5

The CI/NH_3 spectrum of GC peak 8 of demetallated JC5, labelled as "unknown" in the graphic presentation Fig.83, shows quasi-molecular ions at m/z 524 and m/z 538 (Fig.97).
Unexpectedly the isotope patterns of metalloporphyrins appear even after demetallation. They are tentatively interpreted as Cu-C_{31} A-2 and Cu-C_{32} A-2, probably Cu-$C_{31/32}$ DPEP. The distribution of both homologues is comparable to that of the free bases (see Fig.81). Identical components were detected in demetallated JC2, denoted with x in Fig.79, but not in demetallated JC3.

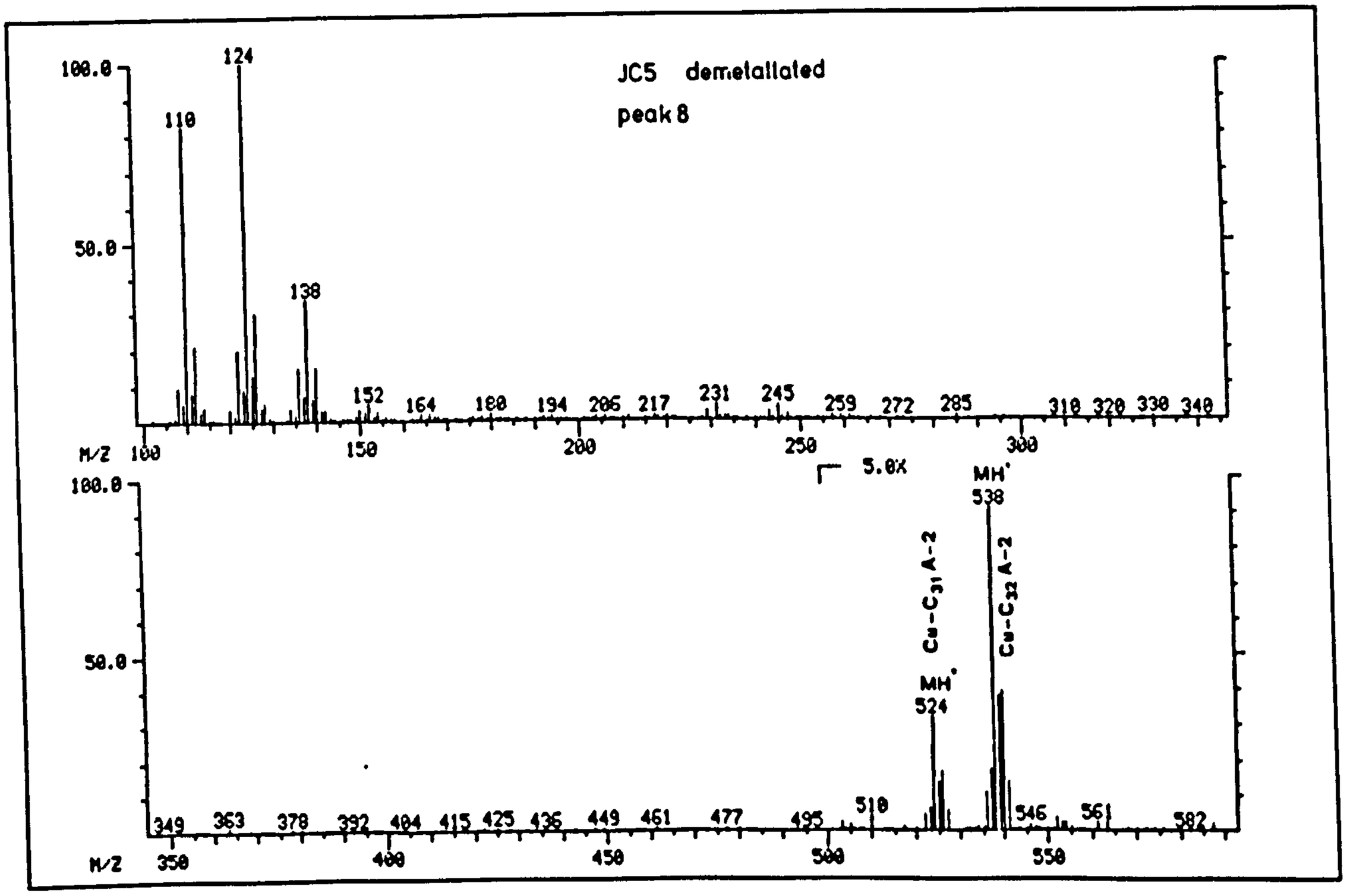


Fig.97 CI/NH_3 mass spectrum of peak 8 of demetallated JC5 (see Fig.82).

Conclusion

Only three structurally defined reference compounds could be investigated in this study. At least from this limited number of data it must be concluded, that monopyrrolic ions have comparable importance as dipyrrolic fragments in the structure elucidation of petroporphyrins by means of CI/NH_3 MS. Although dipyrrolic fragments are commonly considered to be more informative the mono-pyrrolic range contains a similar degree of information, moreover represented by more intense ions.

Any assignment of porphyrin isomers based exclusively on CIMS data is questionable, especially if isomerism occurs on one particular pyrrole ring and therefore the substitution on the three other pyrroles remains unchanged. A comparison of the mass spec-

tra of the two references bearing a methyl-cyclohexyl- or a cycloheptyl exocyclic ring, respectively, exhibits analog mono- and dipyrrolic fragment ions and only little differences in the intensity ratios.

Structurally defined reference compounds are a prerequisite of structure elucidation of petroporphyrin isomers by means of GC/MS. For an unambiguous assignment of individual isomers, particular in mixtures, gas chromatography is superior, even in the age of MS/MS. After the semi-systematic characterisation based on EI-mass spectra and after the tentative structural assignment based on CI/NH_3 measurements, complemented by retention data from gas chromatography, GC/MS of volatile free base and metalloporphyrins appears to be an improvement to direct probe MS (MS/MS), and a recommendable way to elucidate structures of unknown free base porphyrins in the complex environment commonly found in petroporphyrin mixtures.

V.3. Capillary SFC/MS Analysis of Fraction JC5 - JC7

In contrast to the foregoing Sections this Section reports some preliminary results. It demonstrates that even under non-ideal conditions (neither the mobile nor the stationary phase is optimised) capillary SFC/MS is, at least in principle, supplement to high temperature GC/MS in the analysis of metalloporphyrin fractions of shales. It also demonstrates the limitations of capillary SFC/MS which might arise in the analysis of naturally occurring petroporphyrins.

Earlier reports, dealing with the separation of alkylporphyrins by means of capillary SFC, described the use of mobile phases and/or modifiers, which clearly act as CI reagent gases (Jackson, W.P., 1985; Fields, S.M., 1988). One aim of this work is to link the high temperature GC/MS with capillary SFC/MS measurements. Consequently, the same CI reagent gas (NH_3) should be used. This requirement can not be fulfilled by the above cited SFC methods. The mixed, proton free mobile phase system CO_2/CF_2Cl_2 = 30/70 (mol/mol) preferred in this study, is based on Klespers first successful application of supercritical fluid chromatography (Klesper, E. et al.1962), which by chance also deals with porphyrins. The mobile phase mixture acts as a charge exchange reagent gas, allowing the free choice of the CI reagent gases.

Capillary SFC/MS analysis were performed on a chromatographic system consisting of a high pressure syringe pump, modified for pressure control and described in Section VII.2.4., linked simultaneously by the interface described in Section II.2. to the high temperature GC/MS system described in Section III.1..

The CI reagent gas was ammonia. For the analysis of standard metalloporphyrins depicted in Fig.98, a selective scan range between m/z 400-700 and a density program at 120° (see Section VII. 2.4) were chosen.

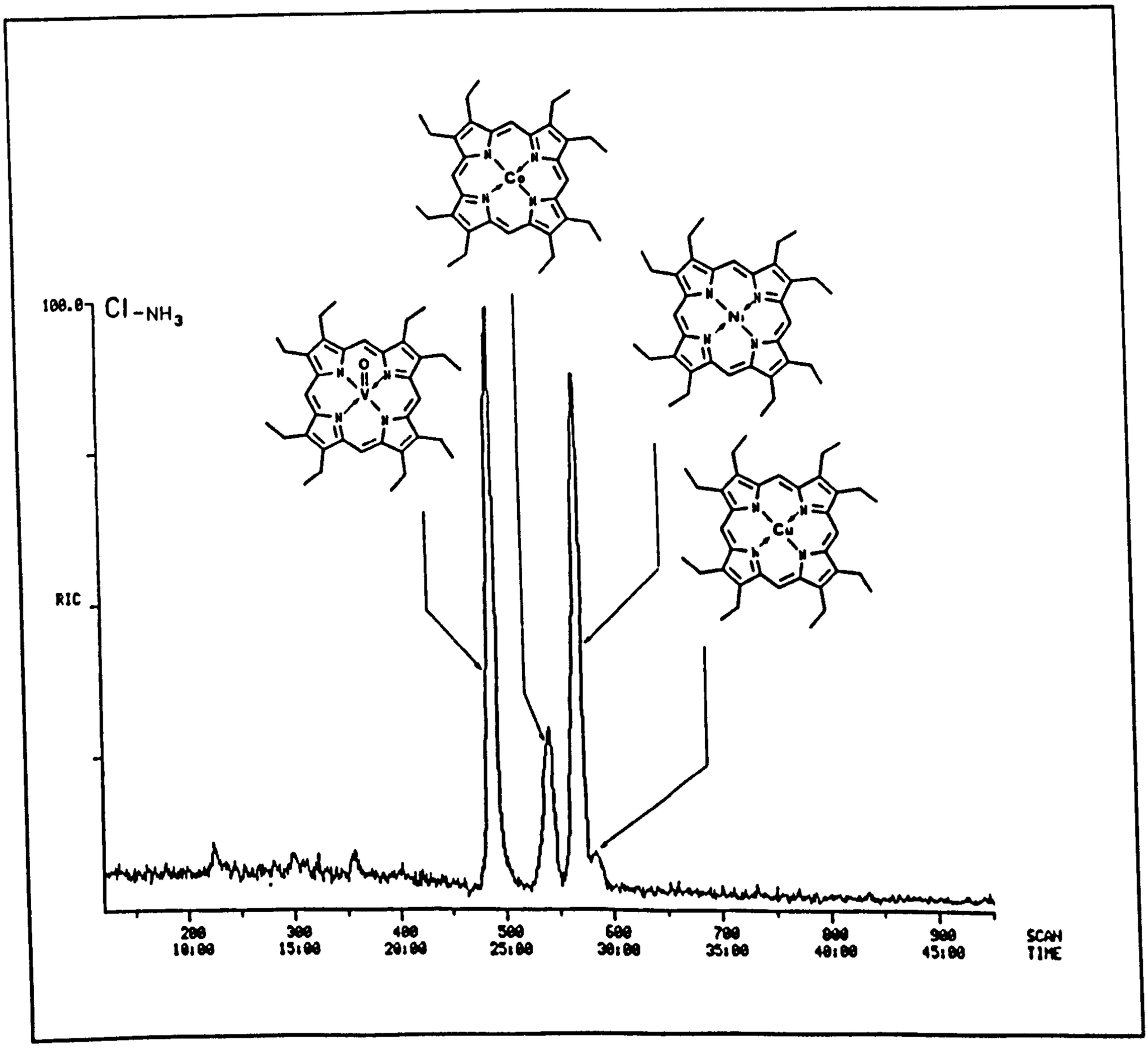


Fig.98 Selected Capillary SFC/MS chromatogram (m/z 400-700) of vanadyl-, cobalt-, nickel- and copper octaethylporphyrin. Mobile phase: CO_2/ CCl_2CF_2; CI-reagent gas: NH_3. For experimental details see Section VII.2.4..

Next we tried to get the link between the upper limit of high temperature GC/MS and capillary SFC/MS. The TIC gas chromatogram of fraction JC5 (Fig.74 represents the very limit of high temperature GC. It suffered from peak broadening and from the loss of several trace components. Therefore, we analysed JC5 again by SFC/MS and hoped for an improved separation efficiency. The result is shown in Fig.99. Very disappointingly, the separation efficiency was not improved to that already obtained in the TIC gas chromatogram.

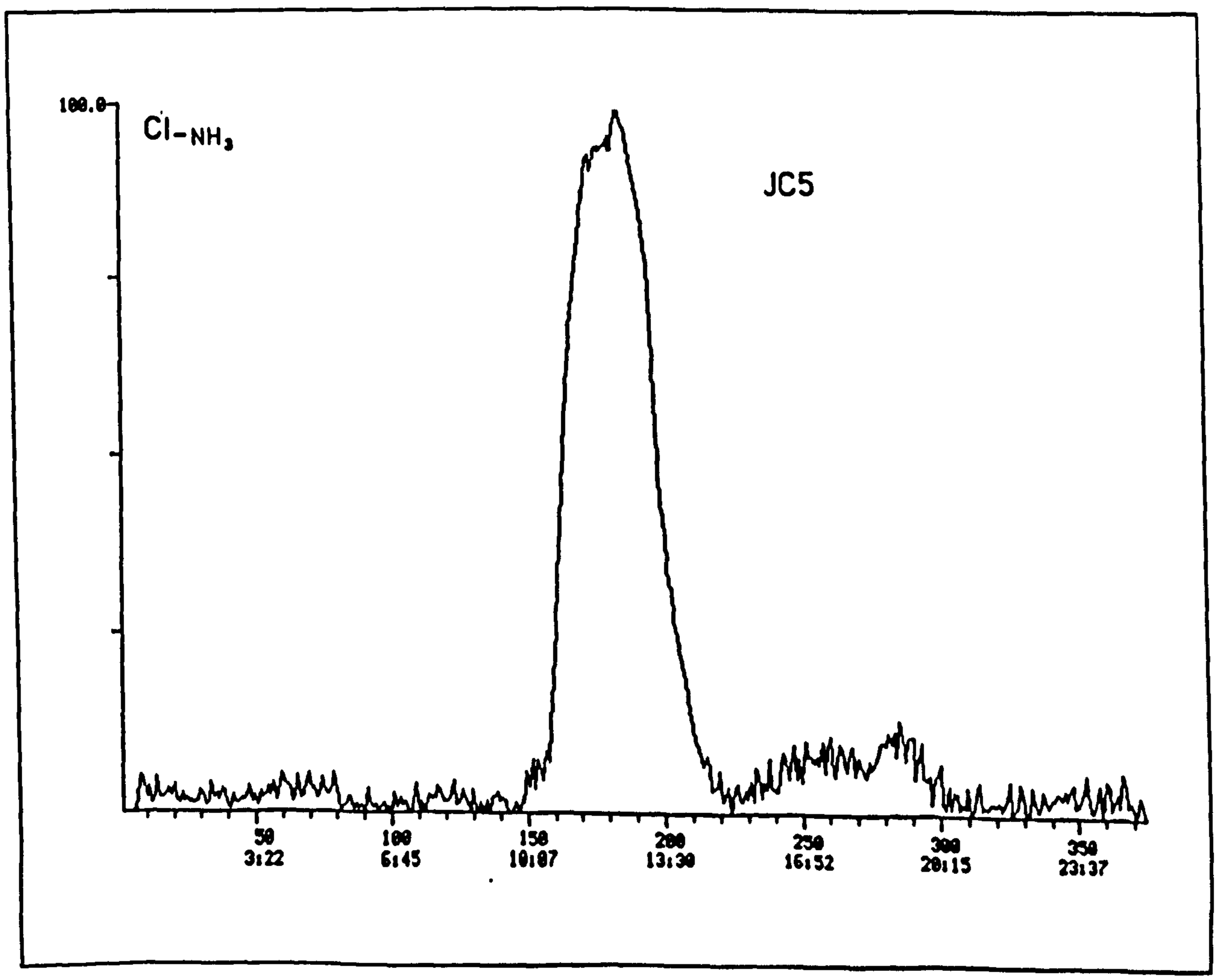


Fig.99 : Capillary SFC/MS selected ion chromatogram (m/z 400-700) of JC5. Working conditions as for Fig.98.

However, the selected summed mass spectrum over the main peak of the SFC/MS chromatogram shows, beside a vanadylporphyrin distribution as already obtained in the GC/MS record of JC5, two additional homologues of higher molecular weight at m/z 554 (V-C_{33} A-4) and m/z 568 (V-C_{34} A-4).

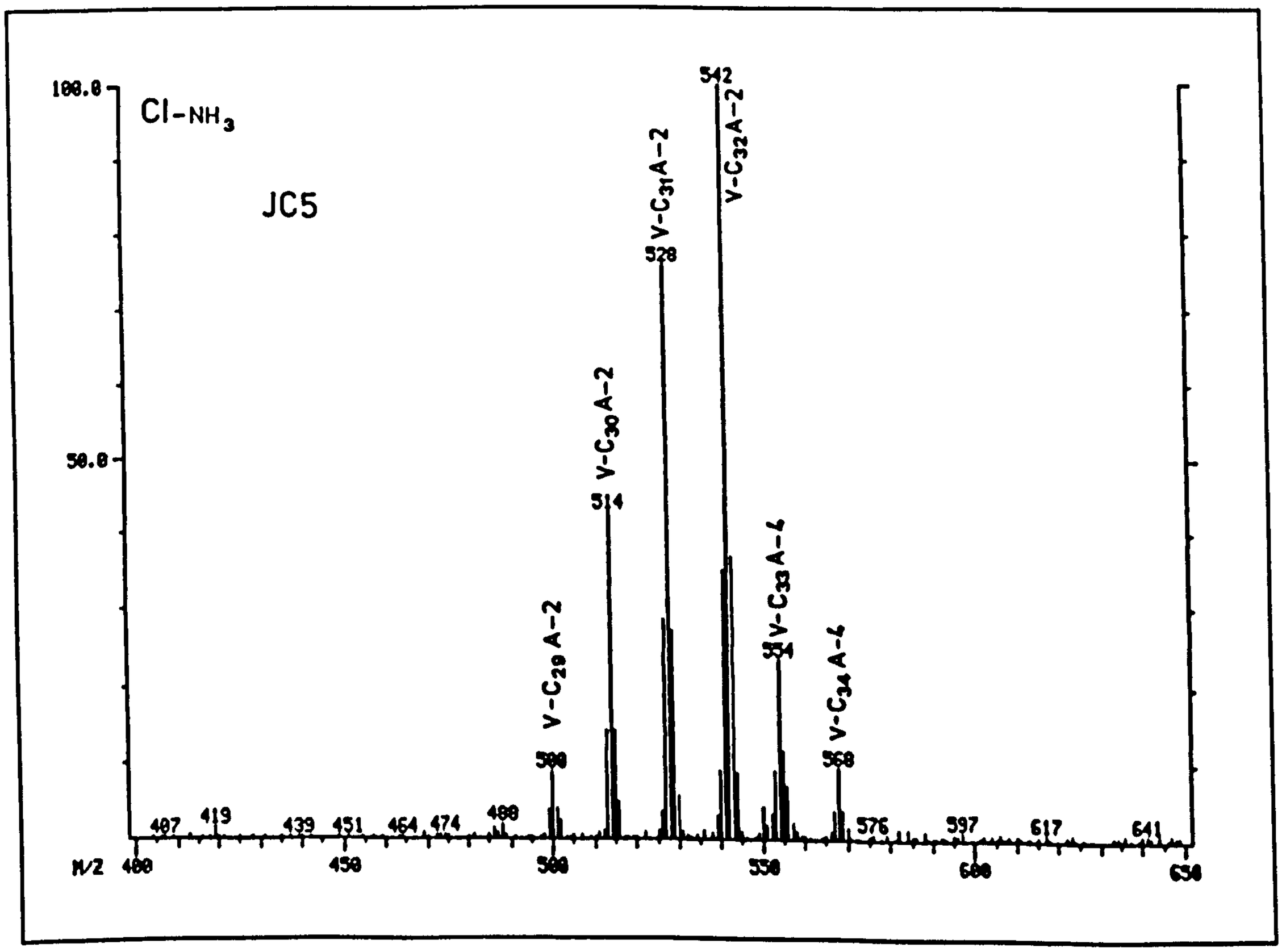


Fig.100 Summed CI/NH_3 mass spectrum of scans 159-207 from mass chromatogram of fraction JC5 shown in Fig.99.

Since our problems with SFC/MS may be of interest in relation to future attempts to analyse petroporphyrins, they are briefly outlined below.

During the SFC/MS analysis of JC5, a decrease in the ion source pressure was observed when the bulk of sample passed the exit of the interface. After several injections the restrictor orifice was clogged. This problem existed even if the restricter was opened to flow rates of more than 10ml/min and heated to 400°C. Furthermore, all clogged restrictors showed some deposited brownish materials over the last few millimetres. With fractions JC6 and JC7 the exit was already clogged after the first injection and further attempts to analyse these two samples failed.

The underlying problem can be traced back to the fact that at least one component of the mixture loses its solubility in the end region of the restrictor, where the supercritical condition of the mobile phase gets unavoidably lost. In order to save the situation at the end of the restrictor, one could think about choosing another mobile phase. Furthermore, it might be necessary to control factors like pressure and the density gradient of the mobile phase, the Joule-Thompson cooling and the compensating heating rate at the restrictor end to a better extent (see Section III.2.).

Thus, a systematic improvement of this analytical problem has to restart with a search for mobile phases which are able to transport polar (and presumably) high molecular weight porphyrins from the separation system into the ion source. Moreover, the single peak in the SFC chromatogram (Fig.99) seems to indicate that any further separation of polar liquid chromatographic petroporphyrin fractions may become very difficult. In such cases it might be useful to introduce in a first step unknown samples in the well known "direct probe"-mode into the MS.

One should seriously consider this possibility and try how far one gets with it, especially in view of the fact that the separation of homologues leaves a great deal to be desired with both, capillary SFC and GC at very high temperatures.

VI. CAPILLARY COLUMN TECHNOLOGY

VI.1. Introduction

Some remarks about the "old fashioned" glass capillary columns preferentially employed in this study seem appropriate.
On-column injection of petroporphyrins followed by temperature programs up to 400° reduce the durability of a capillary column drastically. The average life-time of a high temperature capillary column used in petroporphyrin analyses was just about one week, or 20-30 injections. In the course of this study several hundred capillary columns were prepared and "consumed". This is only tolerable if the columns are laboratory made with cheap raw materials, for it would become prohibitively expensive if the columns have to be bought. However, the financial expenditure is only hypothetical, since high temperature capillary GC columns of optimised selectivity and performance as employed in this work are not commercially available!
This means, that any continuation of this study involves the preparation of high temperature glass capillary columns as a conditio sine qua non. For this reason this chapter is concerned with column technology.
In principle, there are various approaches to capillary column technology. Considering all of them would be confusing any user. Therefore, the next chapter surveys on our approach used in this work and describes the preparation of capillary columns for both high temperature gas chromatography and supercritical fluid chromatography.

VI.2. The Role of Column Technology in the Gas Chromatographic Separation of Geoporphyrins

Whenever a capillary column is used in a more than trivial analytical problem, as in the case of the chromatographic separation of geoporphyrins, it may be of importance to be familiar with the details of its preparation. The deeper knowledge of the inner "life" of a column is a prerequisite in order to achieve optimum separation conditions. This means, one has to choose the most selective stationary phase, the most desirable film thickness or select the best column dimensions (Blum, W.,1988c). Since the applications shown in this work are carried out exclusively on laboratory made columns (which means, that the solution for the separation problems was not bought), the underlying technology of high temperature gas chromatography is of fundamental significance and should be discussed in detail.

VI.3. Column Technology

Coating fused silica capillary columns with an OH-terminated polysiloxane phase of moderate polarity was introduced by Verzele et al. (Verzele, M. et al., 1983), and continued on glass capillaries by K. Grob (Grob, K. et al 1985) and W. Blum (Blum, W. 1985, Blum, W. 1986b; Blum, W. et al., 1987; Blum, W. et al., 1988). The first CH_3O-terminated stationary phase was introduced by Blum and Eglinton (Blum, W. and Eglinton, G., 1989). Compared to coatings based on traditional endcapped phases, the latter authors found as a main advantage an increased thermostability. Pronounced surface bonding of the phase leads to a more inert surface, producing less catalytic destabilisation of the coating.

Whereas Verzele at al. confined their work to an OH-terminated phenylmethylpolysiloxane, Grob and Blum had shown that the technique is also applicable to phases containing phenyl- and cyanopropyl-groups. The apolar phases were introduced by Blum (Blum, W. 1985; Blum, W. et al., 1987). As a general result, the technique is basically applicable to all OH/CH_3O-terminated polysiloxane phases, and provides columns which still exhibit good inertness after routine use above 400°C for apolar or some medium polar phases.

VI.3.1. OH/CH_3O-Terminated Polysiloxane Phases for High Temperature Gas Chromatography

The usual recommendation of laboratory produced stationary phases as described in the literature is not very helpful. Normally the practical working analyst has neither the time nor the facilities or the experience to synthesise polymers. Therefore in this work only polymers are used which are commercially available. The following commercially available phases listed below have been used:

a. PS-347.5 (100% methyl, OH-terminated)
b. PS-089 (95% methyl, 5% phenyl, OH-terminated)
c. PS-086 (85% methyl, 15% phenyl, OH-terminated)
d. PS-090 (80% methyl, 20% phenyl, CH_3O-terminated)
e. OV-61-OH (67% methyl, 33% phenyl, OH-terminated)
f. PS-225-OH (50% methyl, 25% phenyl, 25% cyanopropyl, OH-terminated)

All PS-phases are available at Petrarch System, Inc., Bristol, USA; the OV-phases are available at Ohio Valley Specialty Chemical, Inc., Marietta, Ohio, USA.

VI.3.2. Wettability

Perfect wettability of the support by a given stationary phase is a key feature of coatings with OH/CH_3O-terminated polysiloxanes. With the traditional endcapped phases, it was commonly found that columns were sometimes produced with perfect characteristics concerning bleeding behaviour, thermostability, and inertness, which exhibited a slightly reduced separation efficiency as sole weakness. In other words, the deficient wettability of the support surface resulted in an imperfect, wavy or interrupted coating. The non-ideal film produced some peak broadening without affecting any other characteristics of the column. The opposite is true with OH/CH_3O-terminated polysiloxane coatings. Perfect spreading of the stationary phase on the support surface is a prerequisite for good column quality in general. This is not surprising since coating with these phases is essentially based on a condensation process between silanol groups of the phase and residual silanol groups of the support surface. Obviously, this process occurs with maximum efficiency only when there is full contact between phase and support, i.e. when the support is perfectly wetted.

Fortunately, OH/CH_3O-terminated phases are more easily stabilised as ideal films than their traditional endcapped analogs. This may be due to a slightly reduced surface tension caused by end groups which are, in terms of molecular interactions, incompatible with the bulk of the molecules.

Nevertheless, perfect coating with moderately polar phases involves rather stringent wettability requirements. Minute spreading imperfections which are easily overlooked with traditional coatings cannot be tolerated, since the minute loss of separation efficiency may be accompanied by an obvious loss of thermostability and inertness. So far we have never been able to produce inert and thermostable columns with only mediocre coating efficiency. In contrast, provided perfect wettability of the support has been achieved, higher separation efficiencies are observed

than with traditional coatings. This demonstrates a previously unknown, high degree of film stabilisation owing to surface-bonded polymers.

VI.4. Pretreatment of the Glass Surface

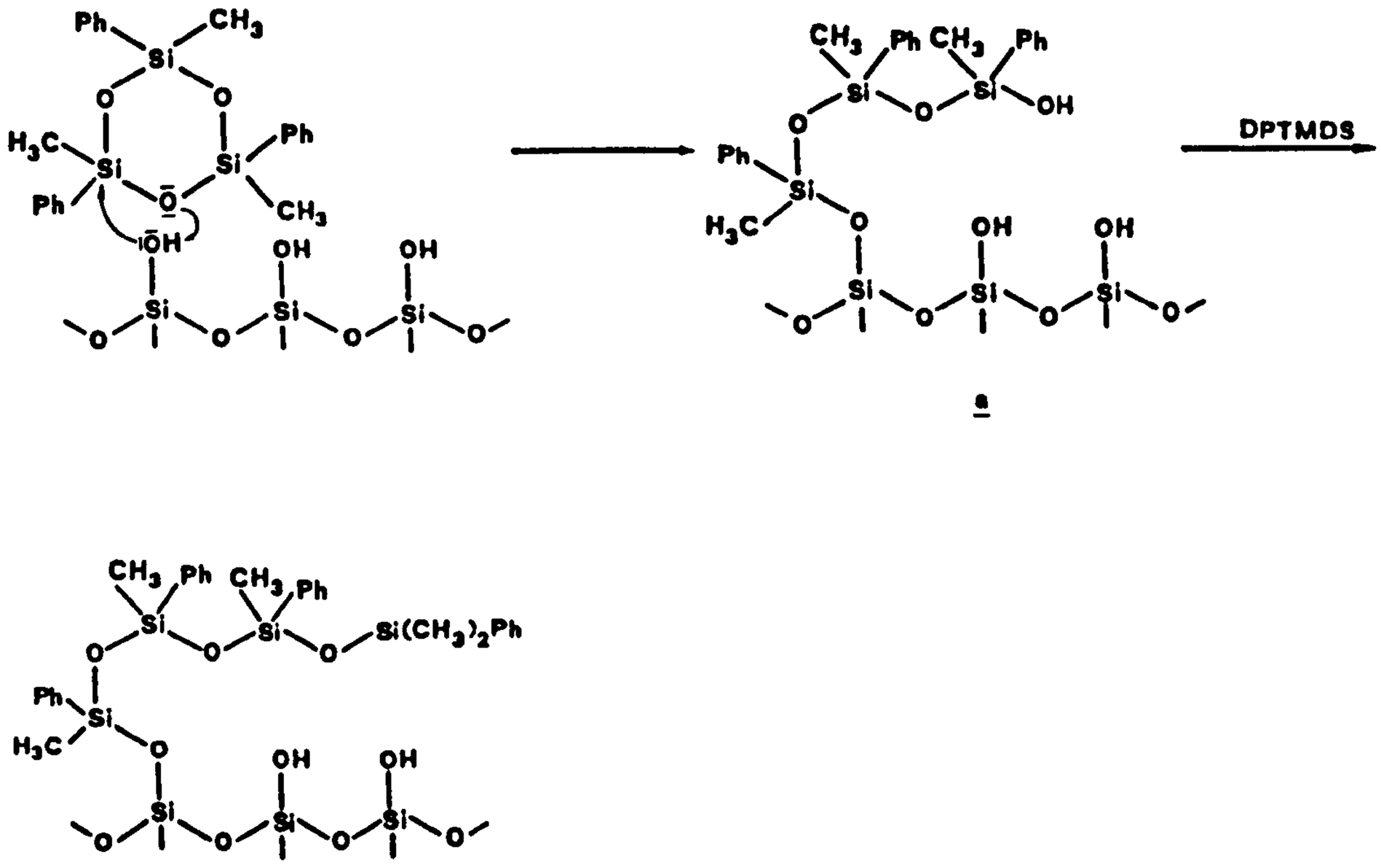
VI.4.1. Leaching (Deionisation)

In contrast to virtually metal-free fused-silica capillaries all types of glass contain metal ions, and some of them covalently bonded boron, acting as adsorptive and chemically active sites. The negative effects of metal ions are commonly eliminated by transforming the internal surface of capillaries drawn from any type of glass into pure silica. The primary purpose of this leaching is deionisation. As a secondary, sometimes desired effect, leaching also increases the density of silanol groups at the surface; these groups may serve as starting point for further surface modifications as well as for chemical reactions with functional groups of a stationary phase. This is of particular importance if OH/CH_3O-terminated polysiloxanes are used as coatings. Acidic leaching and dehydration (see experimental section), followed the methodical instructions given by K. Grob (Grob, K. et al. 1982; Grob, K.,1986a).

VI.4.2. High Temperature Silylation

The required wettability has to be adjusted by modifying the support surface. Persilylation brought almost instantaneous release and redirected effort to an entirely new column type, the inert, temperature-stable column which became the workhorse of present analytical practice. T. Welsch in 1977 (Welsch, T. et al. 1977)

discovered the phenomenon of high temperature silylation. Combining this discovery with deionisation leads to persilylation, introduced by K. Grob et al. (Grob, K. et al., 1979). The history of high temperature silylation was carefully reviewed very recently by T.Welsch (Welsch, T., 1988). Fortunately, it appears that the serious doubt expressed with respect to the feasability of organic surface modification at elevated temperature (Rutten, G. et al., 1984) are proving substantially groundless (see also VI.4.). Numerous silylation processes with structurally specific agents result in wettability effects which correlate perfectly with the organic structure presumed to be built up by the respective agents. The effects could not be explained on the basis of extensive, or almost total, thermal degradation of the organic structure in the course of the high temperature treatment. Consequently, all subsequent ideas are based on the assumption that the final organic surface structure can be directly deduced from the structure of the reagents applied. Optimisation studies carried out so far have shown that, for most of the phases used in this work, roughly the same silylating treatment can be carried out. The practical details as presented in the experimental part (see Section VII.2.7.) schematically followed a description given by Blum (Blum, W., 1985). It is well known that the wettability can be increased only modestly by silylation with monofunctional silylating agents such as 1,3-di-phenyl-1,1,3,3-tetramethyldisilazane (DPTMDS). The reason may be the discrimination of an immobile, sterically fixed, functional group on the support surface, as compared to the corresponding mobile group in the phase whose steric position is constantly being adapted to its environment. The discrimination is reduced, or almost overcome, when more mobile functional groups are bonded to the surface. This can be done by using the cyclic silylating agents introduced by Blomberg (Blomberg, L. et al., 1981), and discussed previously by Blum and Grob (Blum, W. and Grob, K., 1985).We have to start from the assumption that a cyclic siloxane is attacked by a surface silanol (see Scheme 5). The reaction opens the ring. The resulting linear siloxane is bonded to the former surface silanol, while a new silanol group is formed at its opposite end.

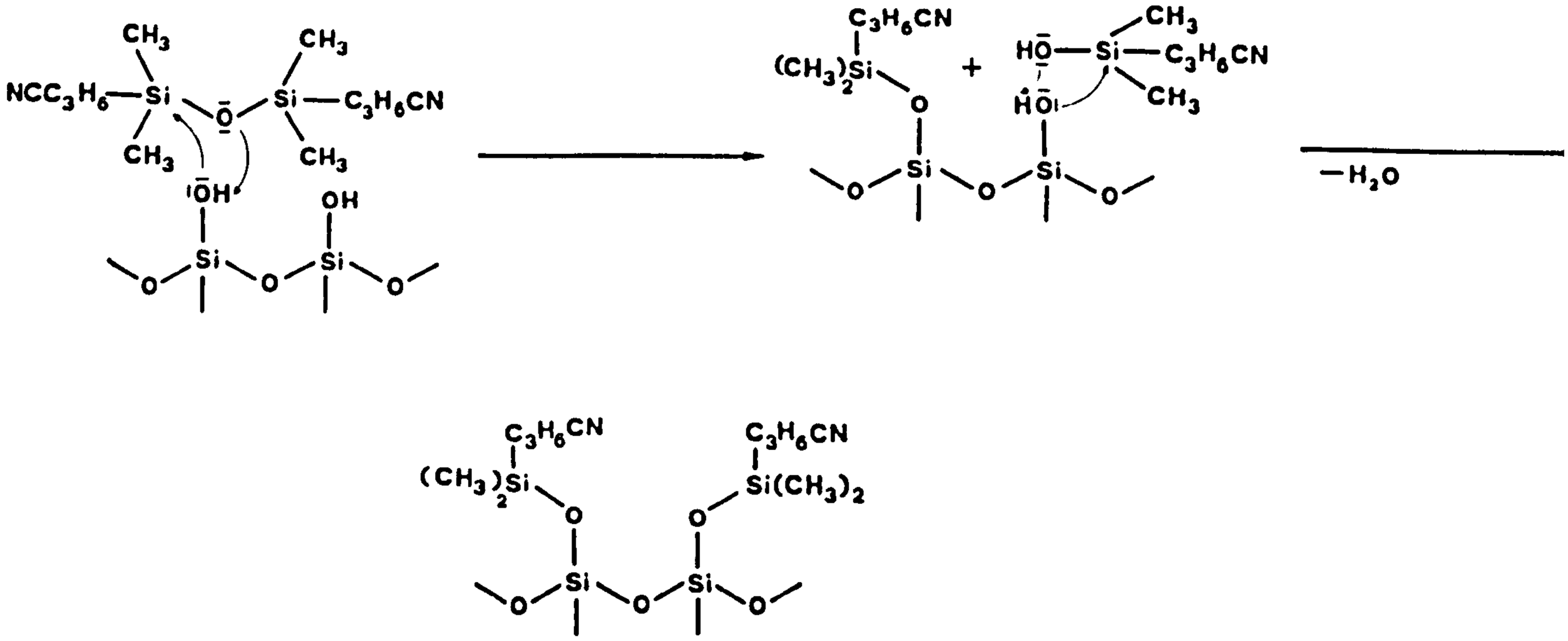


Scheme 5 : Tentative mechanism of the reaction of trimethyltriphenylcyclotrisiloxane (TMTPCTS) with a hydroxylated silica surface. a) Monofunctional reaction with a surface silanol. b) Endcapping with DPTMDS. From Blum 1985

Further reaction with a second surface silanol group, regenerating a cyclic ether, would be possible but we expect that this reaction has a relatively low probability, particularly on a strongly hydroxylated surface. Additional endcapping with mono functional silylating agent such as diphenyltetramethyldisilazane (DPTMDS) provides a means of varying the density of free silanol groups by variation of the reaction temperature. We assume that

high-temperature silylation by thermal degradation of polysiloxanes works similarly in principle, since the primary degradation products are also cyclic siloxanes (Blum, W. and Grob, K., 1985). The main difference is the missing end-capping. The bifunctional reaction of the silica surface is slow, and the monofunctional reaction is highly competitive. Accordingly, as an initial product, short OH-terminated, chains are probably bonded to the surface and continue to react with each other, thus constantly varying in chain length, and probably also undergoing unknown side reactions. In contrast, end-capping immediately stabilises the chains and prevents undesirable side-reactions. This results, on the support surface, in an accumulation of phenylmethyl groups with a mobility similar to that of a free polymer molecule. Such a surface permits more molecular interactions with a stationary phase film than a surface carrying single phenyl-dimethyl or diphenyl-methyl groups.

In order to coat the most polar stationary phase OV-225-OH used in this study a modification of the leached and dehydrated silica surface with bis(cyanopropyl)tetramethyldisiloxan (CPTMDS) according to a method described by Blum (Blum, W., 1986 a, b, Blum, W. et al., 1988a) was used. Compared to treatments using bifunctional silylating agents (Blomberg, L. et al., 1981; Blum W. and Grob, K., 1985) persilylation with CPTMDS could be performed at higher temperatures (380°C) and the treated silica surface showed improved inertness, and an increased density of surface-bonded cyano functions. As a monofunctional reagent, similar to a disilazane, CPTMDS is probably able to react directly with the silica surface to a stable product.

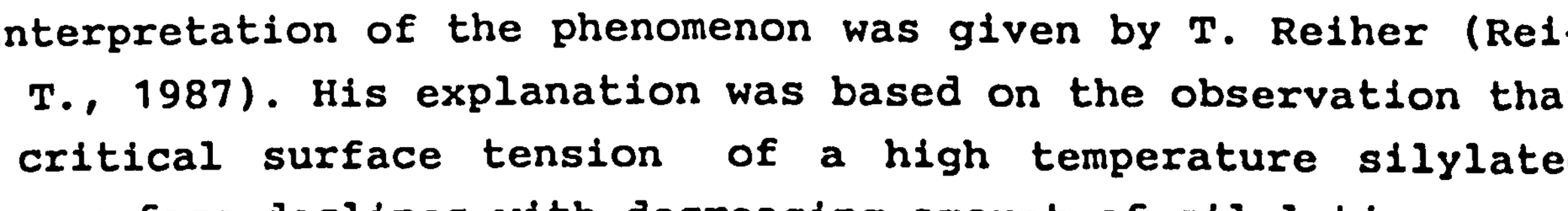


Scheme 6 The mechanism of the reaction of 1,3-bis(3-cyanopropyl)tetramethyldisiloxane with a hydroxylated glass surface. From Blum 1986a

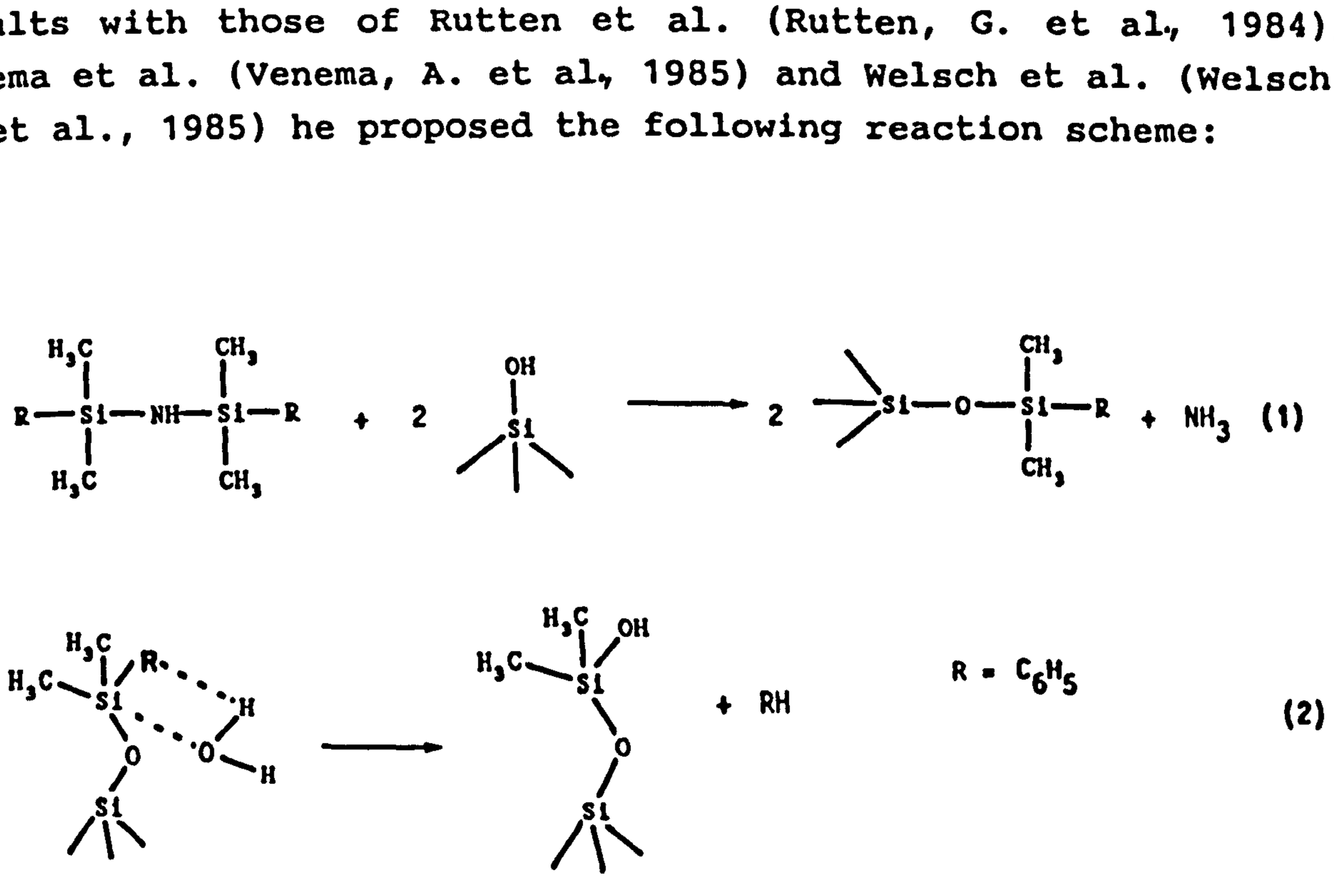
Consequently, it circumvents the various side reactions of cyclosiloxanes (bifunctional after ring opening), which are supposed to react with the silica surface consuming both terminal silanols, and require final stabilisation by end capping (Blum, W. and Grob, K. 1985; Blum, W., 1985). As with the phenylmethyldisilazanes (Grob, K. et al., 1980), CPTMDS yielded a direct correlation of reaction temperature with the degree of silylation.

VI.5. The Condensation Reaction

The relationship between the condensation of reactive terminal functions of a stationary phase with reactive groups of the support surface and enhanced temperature stability was first observed in a systematic comparison of glass- and fused-silica columns coated with medium polar XE-60 (Blum, W. and Grob, K., 1985). However, the reason why surface silanol groups remain even after intensive high temperature silylation, could not be explained. An interpretation of the phenomenon was given by T. Reiher (Reiher, T., 1987). His explanation was based on the observation that the critical surface tension of a high temperature silylated glass surface declines with decreasing amount of silylating agent (1,3-diphenyl-1,1,3,3-tetramethyldisilazane). Correlating his results with those of Rutten et al. (Rutten, G. et al., 1984), Venema et al. (Venema, A. et al., 1985) and Welsch et al. (Welsch, T. et al., 1985) he proposed the following reaction scheme:



(1)

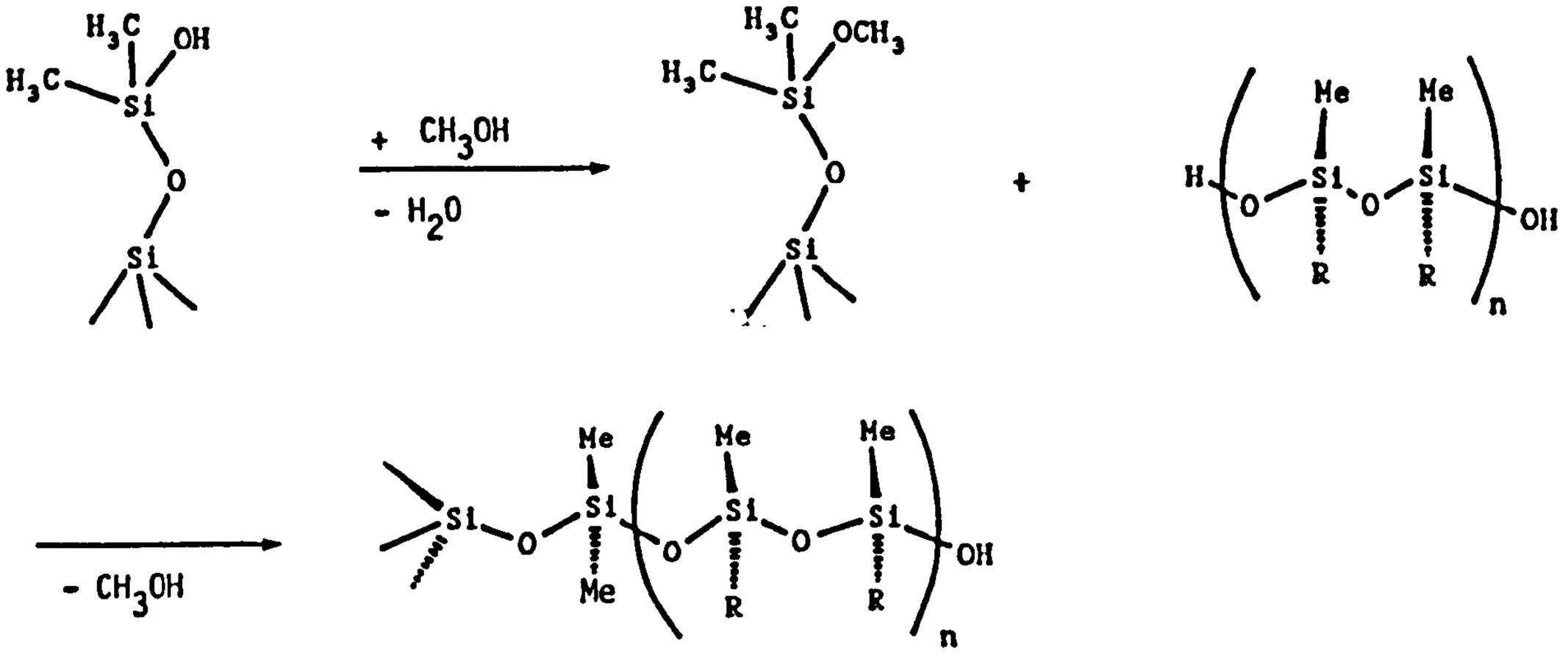


(2)

Scheme 7: Reaction sequence employed by Reiher for the substitution of phenyl groups of a silylated glass surface by traces of water. From Reiher 1987.

In a first step, the expected reaction between silanol groups of the support surface and the silylating agent takes place. In a second step, functional groups of the silylating agent, preferentially phenyl-groups, react with water arising from the glass surface under silylating conditions.
Thirdly, in the original report, a reaction of silanol with surface organosilyl groups is considered, which might be of minor importance, owing to the steric hindrance of this process.

Nevertheless, the second reaction-sequence, the replacement of phenyl-groups by hydroxy-functions, is of fundamental importance, not only for the mechanistic understanding of high temperature silylation, but also for a further optimisation of the method in general. It explains among others (see Welsch, T. 1988) the origin of surface silanol-groups arising after high temperature silylation and the relationship between the density of phenylgroups and newly formed silanol-groups on one hand and silylating temperature on the other hand.
We assumed in a previous report (Blum, W. and Eglinton, G, 1989), that the condensation reaction appears to occur predominantly between the OH-terminated stationary phase and support, and that in situ polymerisation of OH-terminated stationary phase polymers (Verzele, M. et al., 1983) is of minor importance. The same assumption might hold true for the condensation of silanol groups of the stationary phase and the support. Flushing a high temperaure silylated capillary with methanol, as usually recommended (e.g. Grob, K. et al. 1982; Blum, W. 1985), presumably esterifies the silanol groups. Thus, condensation between stationary phase and support takes place between methoxysilane- and hydroxysilane groups forming methanol as a reaction product.



Scheme 8: Tentative mechanism for the condensation of an OH-terminated stationary phase with methoxy groups of the silylated support surface.

Hence, for CH_3O-terminated stationary phases (Blum, W. and Eglinton, G., 1989) the conversion of surface silanol- into methoxy groups should be avoided. It seems that condensation of two methoxysilane (as well as two hydroxy-silane groups) of a polysiloxane needs catalytic promotion, e.g. by an alumina surface (see also Section VII. 2.7.2.) beside the usual heating. The condensation of a CH_3O-terminated phase failed if the excess of silylating agent, which remains after completion of high temperature silylation was removed by flushing with methanol. In this case flushing exclusively with diethyl ether is recommended.

VI.6. The Role of Crosslinking

Today, crosslinking in capillary gas chromatography has become synonymous with advanced column technology. In terms of column quality, surface bonding is the essential step since it provides permanent spreading of the phase, and perfect masking of active sites of the surface. The condensation reaction of OH-terminated phase and methylated surface silanol groups leads to chemical bonding of a part of the phase.

The extent of this reaction depends on the density of silanol groups on the glass surface, the molecular weight of the phase and the film thickness. Following the instructions given in this chapter high temperature stable columns can be prepared even with partially surface bonded films.

Enhanced thermostability is widely attributed to immobilised coatings, although some authors pointed out that up to 350°C column temperature no such improvement could be observed if radical crosslinking was carried out (Blomberg, L. et al. 1983; Grob, K., 1986b). K. Grob called this a "psychological effect of the term bonding" (Grob, K., 1986c).

Additional crosslinking of surface bonded OH-terminated coatings can be performed by flushing the column with aza-tert-butane (Richter, B.E. et al., 1983) as a radical crosslinker (Blum, W. 1985a). After this treatment the coatings are fully immobilised but the temperature stability was found to be the same. For the immobilisation of the hydroxy group terminated stationary phase OV-240-OH, crosslinking with a so-called polyfunctional crosslinker was proposed (Blum, W., 1986b).

Scheme 9: Tentative mechanism for the reaction (cross-linking by condensation) of dimethyltetramethoxy disiloxane with OH-terminated polysiloxane phase. From Blum 1986b.

Crosslinking by condensation at room temperature results in a more inert coating without any decrease of thermal stability (and was carried out for high temperature coatings described in this work). This may have various reasons. In contrast to crosslinking with radical generators (Grob, K., 1979; Richter, B.E. et al., 1983), where vinyl and/or methyl groups form linkages along the chain, the highly specific condensation reaction occurs only at the pertinent site of a given OH/CH_3O-terminated polysiloxane. The system may remain more flexible, and unknown side reactions such as occur with radical generators at elevated temperature may be avoided. In the course of the crosslinking reaction of phase silanol groups with methoxy groups of the crosslinker methanol is formed as a by-product. This may lead in a further step to esterification of carboxyl functions resulting from saponification of some cyano groups of the stationary phase (e.g. OV-240-OH, Blum, W., 1986a and OV-225-OH, Blum, W. et al., 1988a).
For reasons given in Section VI.4., crosslinking by condensation at time is only reported for OH-terminated stationary phases. In general the thermal stability of the immobilised and non immobilised coating was found to be the same up to working temperatures of 400°C, but the columns crosslinked by condensation showed an increased durability. This is of particular interest if, as is necessary for geoporphyrin analysis the full working range of the column has to be used over a long period. We assume that immobilisation by radical crosslinking leads to a more three dimensional structured coating, depending on the extent of crosslinking along the vinyl/methyl groups of the phase. In contrast, crosslinking by condensation only takes place at the OH-terminal groups of the stationary phase resulting in a more linear structure, similar to that of a free polymer.

VI.7. Preparation of Glass-Capillary Columns for Supercritical Fluid Chromatography

VI.7.1. Column Technology

The preparation of narrow-bore, fused silica capillary columns with immobilised coatings, suitable for open tubular supercritical fluid chromatograhy (SFC) as well as high pressure liquid chromatography (HPLC) has been described in several reports (Kong, R.C. et al., 1984a, 1984b; Fields, S.M. et al., 1984; Fokstad, S. et al., 1987). The underlying column technology was derived from preparation methods developed for GC capillary columns. None of the respective reports favoured a particular technology but most of them recommended the use of polysiloxane gum phases in order to achieve a high degree of immobilisation as a prerequisite for the suitability of such coatings in capillary SFC. Because of the small inside diameters the preparation of narrow bore columns is technically not as straightforward as for the wider bore GC columns. One of the most serious technical problems in the preparation of narrow bore capillary columns coated with gum phases is to fill the relatively high viscosity coating solutions into the deactivated capillaries for a subsequent static coating. Owing to this technical limitation, for columns having an i.d. of 50 µm, and (when coated with gum phases) only film thicknesses between 0.2-0.3 µm are described for SFC/MS (Smith,R.D.et al., 1982a, 1982b, 1983, 1984, 1987; Wright, W.B. et al., 1984), although thicker films would be desirable in order to increase the sample capacity of the columns, and in addition to achieve higher capacity factors and better separation efficiencies (Fields, S.M. et al., 1984). In this context, the use of OH-terminated, polysiloxane phases as discussed for the preparation of high temperature stable GC columns (see section before) has certain advantages. Although having the consistency of fluids, OH-terminated polysiloxane phases can be easily immobilised by condensation. Therefore, coating solutions of signifi-

cantly higher concentration can be introduced into the pretreated capillaries in order to produce thicker coatings than those usually applied. Also, as already discussed, the immobilisation process for OH-terminated polysiloxanes differed significantly from the procedures carried out to stabilise conventional C-terminated gum phases. Gum phases require a radical crosslinking using radical generators like peroxides (Grob, K. et al., 1981) or azo compounds (Richter, B.E. et al., 1983, Grob, K., 1986b) leading to a subsequent chemical bonding and crosslinking of the phase. In contrast with OH-terminated polysiloxanes, condensation reaction of the terminal OH-groups with surface silanols and with alkyloxy groups of a polyfunctional crosslinker is the dominant immobilisation reaction (Blum, W. et al., 1987). Radical crosslinking leads to a three-dimensionally structured coating, depending in the extent of crosslinking along the methyl/vinylgroups of the stationary phase backbone. As pointed out in the previous section, crosslinking by condensation only takes place at the respective OH-terminated groups of the phase resulting in a more linearly structured film, similar to a free polymer chain. The importance of the flexibility of the polysiloxane backbone in order to preserve good solute diffusion and therefore high chromatographic efficiency has been pointed out (Lee, M.L. et al., 1986). For the reasons discussed above, their flexibility is preserved, when coating is carried out with OH-terminated phases. This flexibility persists even after intensive immobilisation and even after the condensation reaction is followed by an additional radical crosslinking, as already described (Blum, W., 1985) and carried out in this work.

VI.8. RESUME AND OUTLOOK

The original aim of this study was to demonstrate the significance and the limit rather than the very end of the application of high temperature capillary gas chromatography in combination with mass spectrometry supplemented by capillary SFC/MS.
The capillary column technology of both chromatographic methods and their interfacing to MS are the principal items of this work. Petroporphyrins have been chosen as target compounds almost by chance, owing to their extreme complexity and their high retenon temperatures, but not in view of their geochemical relevance.

Beside the petroporphyrins of Julia Creek oil shale, reported in Section V, various other oil shales were examined preliminary for this particular purpose, e.g. Serpiano-, Marl Slate-, Green River- and Messel shale. Julia Creek was analysed thoroughly, since it appeared to be the most difficult sample, containing only small quantities of nickel- but mainly vanadyl porphyrins, thus challenging the analytical requirements to the highest extent.
It was not attended to compare the analytical techniques or the results with other common methods and their results, respecively. The danger of misinterpretations would be high because the work in this thesis is unique in measuring metalloporphyrins directly. Furthermore, a comparison is hampered by the fact that just one oil shale has been analysed in detail.
Concerning the instances where common procedures were used, we want to add some comments.
This study was carried out on only 11g pulverised Julia Creek shale. Necessarily, the sample was pretreated (e.g. demetallated) on a comparable small (micro) scale. It may be due to this restriction, that a part of the sample appeared to degrade during the demetallation with methanesulfonic acid or - for unknown reasons - was chelated to copper porphyrins. This way, we lost two metalloporphyrin fractions, which had be sufficiently analysed before the treatment with methanesulfonic acid. The question re-

mains open as to the working procedure has been inadequate or whether the demetallation suffers from decomposition in general. If partial degradation is occuring generally, then it might be that the higher unsaturated petroporphyrins are more sensitive to strong acids.
The gas chromatographic analysis of geoporphyrins at the maximum working temperature of the method is, in fact, a border-line application. Since petroporphyrin mixtures contain both homologues and isomers, different polar coatings of the capillaries are required.
Homologues separate according to their retention temperatures; therefore they are resolved best on apolar columns, when they appear at comparably low retention temperatures. In contrast, isomer separations require selective, medium polar coatings, bearing phenyl functions. Since with increasing polarity the retention temperatures shift to significantly higher values, low retention temperature and high selectivity, cannot be met by one type of column. Accordingly, further optimisation has to restart with the improvement of homologue separation and this problem leads directly to multidimensional chromatographic methods.

It was shown on selected examples that capillary SFC/MS supplements high temperature GC/MS, particularly in the analysis of metalloporphyrins. However, SFC/MS did not come up to expectations and did not improve homologue separation. Nevertheless, the combination of capillary chromatographic techniques with mass selective detection in both, EI- and CI-mode, led for the first time to the direct analysis of underivatised free base- and metalloporphyrins. The insufficient resolution power of high temperature GC as well as of capillary SFC has overridden the potential of chemical ionisation to such an extent that also CIMS did not come up to expectations. This problem was enhanced by secondary reactions in the ion source.
Although the techniques described in this study represent the present state of the art in direct analysis of petroporphyrins by means of high temperature capillary GC- and SFC/MS, many questions of the analytical problem "structure analysis of geoporphyrins", remain open, and this work is only a little step forward.

The methodology offered is still far from a full resolution of the complex mixture situation met in any oil shale extract. Almost each "resolved" GC-peak still conceals a variety of individual geoporphyrins, and it was nearly impossible to correlate structurally significant fragments to proper molecular ions. This means that a systematic improvement of geoporphyrin analysis has to restart with an enhancement of the chromatographic resolution power, in particular with that of the homologue separation.
Improvements of stationary phase selectivity are imaginable, but the possible working range will approximately be limited to the values demonstrated in this study. Any extension of the application range to higher molecular weight porphyrins appears to be difficult and will need shorter columns involving a loss of separation efficiency.
Multidimensional chromatographic methods e.g. by taking advantage of apolar and polar capillary columns, or the pronounced selectivity of HPLC and the comparably high separation efficiency of capillary GC (or SFC), directly combined to a mass spectrometer, may be a further step forward in order to simplify the complexity of geoporphyrin mixtures. The technical prerequisites are given. Transfer of selected fractions from HPLC into the inlet of a capillary column has been described and HPLC-GC combinations are commercially available. The final step, the coupling of the high temperature capillary GC to the MS has been described in this study .
A further logical extension could be worked out on the detection side by improving the selectivity of the mass spectrometer. Two-dimensional MS (MS/MS) allows the selection of distinct "parent-ions" (e.g. molecular ions), their subsequent fragmentation by collision and the production of "cleaner" (secondary reaction free) mass spectra. Unfortunately, this very attractive alternative will be of limited value as long as classical CI, as employed in this study, is used. The fragmentation of porphyrins under CI conditions to structurally significant mono-, di- and tripyrrolic fragment ions results from the degradation of respective porphyrinogen- $(M+6+H)^+$ ions, which are only of minor importance in CI-spectra obtained by GC/MS. In all CI-spectra, independent of the proton affinity of the chosen reagent gas, $M^{+\cdot}$-

ions or the MH^+-quasi-molecular ions dominate but both do not participate directly in any fragment formation. In other words, a possible success of MS/MS in the mixture analysis of petroporphyrins depends above all on chemical ionisation conditions which supply a high yield of porphyrinogen ions.

This work reveals quite clearly the system-inherent limitations of both GC/MS and SFC/MS working with classical ionisation methods under reduced pressure. (i) The design of commonly used mass spectrometers requires relatively long transfer lines (ca. 30 cm), which lead to a loss of chromatographic resolution at retention temperatures above 360°C. (ii) The reduced pressure in the ion source causes an early decline of the density of a supercritical fluid along the interface line, and (iii) secondary reactions in CI ion sources appear to be unavoidable.

One possible way out may be the use of chemical ionisation at atmospheric pressure (API), producing only quasi-molecular ions and restraining any fragmentation (subsequent fragmentation of selected quasi-molecular ions occurs in the collision chamber of a MS/MS system). In addition, the chromatographic system has not to be coupled to vacuum and the distance between ion source and column is comparably short. Thus, a heated transfer line is not longer necessary.

In other words, an improvement of direct petroporphyrin analysis also points to an other type of mass spectrometer than that used in this study.

The development of new techniques is nowadays often involved with commercial aspects. Hence, capillary SFC is sometimes put forward as being a substitute rather than an extension of high temperature GC. This study clearly shows the advantages but also the inherent limitations of the two capillary chromatographies. The work demonstrates that both methods should be used in a complementary fashion, against the spirit of this age in analytical chemistry. The common grounds can not be overlooked:

Both methods are based on the same column technology and at last can be coupled to a common spectrometer.

VII. EXPERIMENTAL

VII.1. Sample Preparation

VII.1.1. Preparation of Hexahydrooctaethylporphyrin (see Fig.2)

2mg of octaethylporphyrin was dissolved in 5ml dichloromethane in a 10ml vial (Pierce vial) and flushed, under stirring, with argon for 10 min. 200mg Raney-Ni/Al in ethanol was added and argon replaced with hydrogen. After 10 min, the solution was nearly colourless. The vessel was centrifuged, the solvent decanted under argon protection and immediately used for GC and GC/MS analysis, respectively. The yield was about 70%.

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VII.1.2. Preparation of Dimethyl-silicon(IV)octaethylporphyrin (see Fig.3).

2mg octaethylporphyrin was dissolved in 1ml dry toluene in a thick-walled glass vial (Pierce vial). 2ul dichlorodimethylsilane was added and the mixture heated at 130° for 5h. Aqueous KOH (2N) was than added until all solids were dissolved, and extracted twice with 5ml dichloromethane. After drying the organic phase with Na_2SO_4 the solution was concentrated to a volume of 1ml and used for GC/MS analysis. The yield was about 2%.

VII.1.3. Preparation of Metallo Octaethylporphyrins (see Fig.5)

The single metalloporphyrins were prepared from free base octaethylporphyrin. Free base octaethylporphyrin was purchased from Fluka AG, CH-9470 Buchs, Switzerland.

VII.1.3.1. Nickel(II)/Co(II)-octaethylporphyrin

The insertion of nickel into octaethylporphyrin followed a method of Fuhrhop et al. (J.H. Fuhrhop et al., 1975). 4mg free base porphyrin was dissolved in 3ml chloroform and 1ml saturated glacial acetic acid, copper(II)acetate solution (available at Ventron-Alfa Products, D-7500 Karlsruhe, W-Germany) was added. The mixture was refluxed for about 5h, diluted in about 10ml of dichloromethane and washed 3 times with 15ml water. After drying the organic phase with Na_2SO_4, the solution was concentrated to a volume of 5ml and analysed by GC. Since this sample of Ni(II)acetate contains about 10% of Co(II)acetate as an impurity Co(II)-octaethylporphyrin was formed as a by-product. The yield of the metal insertion was 100%.

VII.1.3.2 Copper(II)octaethylporphyrin

Copper insertion into octaethylporphyrin was carried out as for nickel. Copper(II)acetate was purchased from Ventron-Alfa Products, D-7500 Karlsruhe, W.-Germany. The yield was 100%.

VII.1.3.3. Vanadium(IV)octaethylporphyrin

Oxovanadium(IV)octaethylporphyrin (vanadylporphyrin) was prepared by a method of Erdmann et al. (Erdmann, J.G. et al., 1956). 4mg free base octaethylporphyrin, 80mg oxovanadium(IV)sulphate (available at Ventron-Alpha Products, D-7500 Karlsruhe, W.-Germany) and 10mg sodium acetate were dissolved in 5ml glacial acetic acid and 1ml dry pyridine. After reflux at 160°C for 5h the mixture was poured into 10ml water and extracted 3 times with 10ml dichloromethane. The organic phase was dried with Na_2SO_4, concentrated to a volume of 5 ml and analysed by gas chromatography. The yield of metal insertion was 100%.

VII.1.4. Extraction of Sediments

Pulverised Julia Creek oil shale (11g) was extracted with 200 ml chloroform for 24h in a Soxhlet extractor. The solution was reduced to dryness under vacuum. The yield of organic residue was 94.12mg.

VII.1.5. Demetallation of Metalloporphyrins (JC2, JC3 and JC5)

The demetallation of the liquid chromatographic fractions JC2, JC3 and JC5 was carried out according to the reaction conditions described by Chicarelli (Chicarelli, I., 1985). 200ul methanesulfonic acid (MSA, 98%) was added to the single fractions under argon atmosphere. The mixture was heated in an oil bath, at 80°C for the nickelporphyrin fraction JC2 and at 100°C for both vanadylporphyrin fractions JC3 and JC5, over a period of 60min or 120min, respectively. After heating, the mixture was diluted with water and decanted through a filter and extracted with dichloromethane. The organic layer was neutralised with saturated sodium bicarbonate solution, dried with sodium sulfate, evaporated and taken up in 100ul toluene. The toluene solution was purified over 1g silicagel (in a pasteur-pipette) with 5% acetone/toluene and used for subsequent GC/MS analysis.

VII.2. Instruments, Materials and Tools

VII.2.1. Gas Chromatography

The GC analyses of metallo-, free base porphyrins were performed on a high temperature version of a Mega gaschromatograph (CARLO ERBA) HRGC 5300-HT equipped with a constant flow/constant pressure regulator (CP-CF 516) in order to keep the carrier gas flow contant over the whole temperature range.

VII.2.2. Mass Spectrometry

Mass spectrometry was carried out using a Finnigan 4600 quadrupole mass spectrometer with enhanced pumping capacity [two Varian M-4 oil diffusion pumps (pumping capacity 8001/sec/unit), two Edwards E2M40 rotary pumps (pumping capacity 6001/sec/unit; leak rate of the MS-system >10ml H_2/min)] additonally equipped with a CI reagent gas inlet described earlier (Blum, W. et al., 1977). For electron impact- and chemical ionisation the following conditions were employed.

EI-mode

Ion source temperature: 200°C
Electron energy : 70 eV
Emission current : 500 uA
Scan time : 2 sec/cycle
Scan range : m/z 100-700 or m/z 400-700, respectively.

CI-mode

Ion source temperature: 140°C
Electron energy : 70 eV
Emission current : 500 uA
Scan time : 2 sec/cycle
Scan range : m/z 100-700 or m/z 400-700, respectively.
CI-reagent gases : H_2, CH_4, NH_3
The CI-reagent gas pressure was focussed on the highest possible sensitivity.

VII.2.3. Combined High Temperature Gas Chromatography Mass-Spectrometry

High temperature GC/MS analyses were performed on a CARLO ERBA 4160 gas chromatograph equipped with a constant pressure/constant flow regulator (CP-CF 516), linked by the interface described in Section III.1 to the mass spectrometer described above. Special attention has been paid to the deactivation of the glass interface capillary.

VII.2.3.1 Deactivation of the Interface Glass Capillary Line

The interface line was produced on a glass drawing machine and had an outer diameter of 0.8mm, an inner diameter of 0.2mm and a length of about 35cm. The small inside diameter provides sufficient restriction to allow a change of capillary columns without turning off the vacuum system of the spectrometer. The glass capillary line was deactivated using the pretreatment described for glass capillary columns coated with apolar phases (Blum, W., 1985; Blum, W. et al., 1987).

For high temperature work (interface temperature > 380°C) the glass line was additionally coated with a thin film of PS-089 (OH-terminated 95% methyl, 5% phenyl; Blum, W. et al., 1987). All deactivation steps involving heat treatment (leaching, dehydrating, silylating, and chemical bonding of the phase) were carried out in a high temperature tube oven of 60cm length (type RoK 3/60, Heraeus, D-6450 Hanau, West Germany).

The additional coating of the deactivated glass line was effected dynamically by pushing a plug of the coating solution (containing 5% of the stationary phase) through the deactivated capillary line at a rate of 0.5cm/s. Nitrogen was used as flushing gas. After coating, the interface was conditioned at 300°C for 4h, again using nitrogen as flushing gas. Then, a part of the stationary phase was chemically bonded to the support (for details see Section VII.4.1.4). After installation of the interface in its oven and final positioning in front of the ion source, the straightened opposite end of the glass interface line extended

into the GC oven for about 10cm. This end was bent cautiously with a micro-torch to a right-angle. Finally, the capillary column was butt-connected with the interface line (both having the same outer diameter) using a special high temperature stable Teflon shrink tube (PTFE shrink tube, AWG24SW, shrinking ratio 2:1, Otto Pfenninger AG, CH-8712 Staefa, Switzerland). This connection was gas-tight up to an oven temperature of 400°C over several weeks. The two capillaries were separated by carefully peeling off the Teflon tube with a new razor blade.

VII.2.4. Capillary Supercritical Fluid Chromatography

The supercritical fluid chromatograph used in this work consists of a high pressure syringe pump (Model 8500, Varian, Palo Alto, USA) which has been modified for pressure control (Chester, T.L., 1984). The pressure was programmed using a Hewlett Packard 75C micro computer. The syringe pump was combined via a stainless steel transfer line (i.d. 0.1mm) to a HRGC Mega gas chromatograph 5160 (CARLO ERBA) which acts as a constant temperature oven. The injector was an electrically actuated internal loop valve with o.1 or O.2 ul injection volume (Model EC 14W.11, Valco, Houston, TX, USA). Room temperature on column sampling was used. The injector and the restrictor were placed on the top of the GC oven. This chromatographic system was combined with the micrometer adjustable glass interface described in Section II.2. to a Finnigan 4600 capillary gas chromatography/quadrupole mass spectrometer, which had been modified for high temperature capillary GC as described in Section III.1.

Both TIC-SFC chromatograms shown in Figs.98-99 were recorded under the following conditions:

a. Column temperature : 120°C

b. Mobile phase : CO_2/CF_2Cl_2 = 30/70

b. CO_2 density program: 0.18g/cm^3 with a program rate of 0.02g/cm^3/min to 0.5g/cm^3 and with a rate of 0.005g/cm^3/min to 0.69g/cm^3 (400 bar).

VII.2.5. Medium Pressure Column Liquid Chromatographic Fractionation of Julia Creek Crude Extract

The crude extract (94.12mg) was fractionated by means of medium pressure liquid chromatography (MPLC) eluting with solvents of increasing polarity. The separation was performed on a 460 x 26mm column (Büchi-system B-680) filled with silicagel 60 (15-25um, Merck). In order to improve the separation of individual groups (JC1-JC8) the gradient was increased stepwise with a flow of 20ml/min:

1. 200ml hexane
2. 360ml hexane:CH_2Cl_2 = 8:2
3. 480ml hexane:CH_2Cl_2 = 6:4
4. 300ml hexane:CH_2Cl_2 = 5:5
5. 500ml CH_2Cl_2
6. 640ml methyl-tert-butylether
7. 400ml methyl-tert-butylether:ethanol = 5:5

The detector was a deuterium lamp.

From the single fractions JC2 -JC7, disolved in CH_2Cl_2, UV-visible spectra were measured (see Table 2). The spectra were obtained on a Shimadzu U-240 recording spectrophotometer.

VII.2.6. Inductively Coupled Plasma Mass Spectrometry (ICPMS)

The ICPMS analysis of the single liquid chromatographic fractions JC2, JC3 and JC5 were performed on a VG Plasmaquad 1.
For this purpose the samples were first dissolved in 500ul acetone, an aliquot of 100ul taken in a Teflon bomb (Tölg-bomb) and evaporated to dryness. The residues were dissolved again in conc. HNO_3 and heated at a temperature of 165°C over night (Carius-disclosure).The sample introduction was carried out by a spray gun (De Galan spray).

In order to increase the sensitivity a limited mass range between 46.95 - 70.34 a.m.u. was chosen. This range covers the mass region where the transition metal elements, commonly considered to be chelated by petroporphyrins, appear. For the detection a multichannel-analyser with a cycle time of 250usec/channel was used. The system was calibrated by external standards.

VII.2.7. Preparation of Glass Capillary Columns for Gas Chromatography

VII.2.7.1 Preparation of High Temperature Stable Columns (max. Working Temperature ca. 420°C)

As mentioned in section 1.3.2.2 only four commercially available OH-treminated polysiloxanes [PS-347.5 (100% methyl, OH-terminated); PS-089 (95% methyl, 5% phenyl, OH-terminated); PS-086 (85% methyl,15% phenyl, OH-terminated) and PS-090 (70% methyl, 20% phenyl, CH_3O-terminated)] are usable as stationary phases at working temperatures above 400°C. Since all polymers as received from the supplier are fluids with molecular weights between 700 - 10000 dalton, additional polymerisation is necessary in order to improve the thermostability of the phase.

VII.2.7.2 Increasing the Viscosity of Commercially Available Pre-Polymers

In many cases the condensation reaction of OH-terminated and CH_3O-terminated polysiloxane pre-polymers is greatly promoted by an alumina surface (Blum, W., 1985). Any kind of a small pan or vessel of pure aluminum may be used. The internal surface of the vessel has to be thoroughly cleaned with steel wool, washed with diluted NaOH and HCl, and heated in a Bunsen flame. 10gr of the pre-polymer is poured into the vessel which is than heated in a gas chromatograph oven. The heating period and temperature depends on the viscosity of the pre-polymer. The three OH-termina-

ted polymers with a molecular weight of about 10000 dalton were heated for about 1h at 200°C, the CH_3O-terminated PS-090 with a molecular weight of only ca. 700 dalton was heated for 4h at 200°C and for 2h at 250°C. Every 20 - 30 min the material is quickly stirred with a Teflon spatula. After this treatment all phases still have the viscosity of fluids but tend to form strings rather than drops, from a spatula.

VII.2.7.3. Acidic Leaching (Deionisation)

Leaching of borosilicate glass capillaries is a process which requires a modification to the conditions of coating with OH- or CH_3O-terminated phases. For the four phases discussed in this section the ideal depth of leaching is attained by a treatment at 160°C. For this purpose Duran 50 (Schott-Ruhrglas, D- Mainz, Germany) capillaries (i.d. 0.3mm) are filled with 20% HCl, and than flame sealed under vacuum [7% of the length of the column is emptied 93% remains filled]. After keeping at 160°C for 8h, the columns are rinsed with twice the column volume of 1% HCl, and dried for 2h at 280°C, with both ends connected to vacuum.

VII.2.7.4. High Temperature Silylation

The silylation mixture consists of 1vol of 1,3,5-trimethyl-1,3,5-triphenylcyclotrisiloxane and 1 vol. of 1,3-diphenyl-1,1,3,3,-tetramethyldisilazane . 1 vol. of this mixture is diluted with 1 vol. of dichloromethane. The solution is sucked into the capillary to fill about 1.5m (5 coils). This plug is moved through the column with nitrogen at a rate of about 1cm/sec.. As soon as the plug has passed the column, the residual dichloromethane is evaporated by flushing the column for about 30 min. with nitrogen at a pressure of about 1bar. Then both end pieces are carefully flame sealed. Next to leaching, the silylating temperature greatly influences the final concentration of remaining silanol groups on the support surface (see Section VI.4.). This has to be optimised for a given OH- or CH_3O-terminated phase.

However, due to additional polymerisation described in section 7.4.1.1 and owing to the narrow range of polarity of the four high temperature phases, the same silylation temperature (380°C) and reaction time (10h) can be used.
After cooling and opening, the silylated capillaries are rinsed further with methanol and than ether prior to a subsequent coating with the OH-terminated phases PS-347,5; PS-089 and PS-086. For reasons discussed in Section VI.4. they were flushed with ether only, when coated with the methoxy terminated polymer PS-090.

VII.2.7.5. Coating and Condensation

The film thickness of a coating is of some importance for example it significantly influences the durability of a column owing to its masking properties. For all high temperature stable columns a filmthickness of 0.15um has been chosen.
The freshly-prepared coating solutions contain 0.2% of the respective stationary phase dissolved in pentane/dichloromethane (Kong,R.C. et al.,1983; Grob,K.,1985). On completion of the coating , the column was mounted in a gas chromatograph and flushed with hydrogen as carrier gas, programmed at a rate of 4°C/min to an end temperature of 300°C. This temperature was held for 6h. The column was than programmed at the same rate to 350°C and held there for another period of 6h.

VII.2.7.6. Preparation of OV-225-OH Coated Columns

The preparation of the OV-225-OH coated columns followed methods described previously (Blum, W., 1986a; 1986b; Blum, W. et al., 1988a). Leaching temperature (170°C) and the conditions for high temperature desactivation with bis(cyanopropyl)tetramethylsiloxane (Petrach Systems) differes from the pretreatment described above. For silylation the temperature was maintained for 12h at 350°C. As a polyfunctional crosslinker added to the coating solution (1% of the weight of the stationary phase) cyanopropyltriethoxysilane was used. The respective mechanism and details of crosslinking by condensation was disscussed in Chapter VI.3..

VII.2.8. Testing Capillary Columns

Preliminary information on the quality of freshly prepared columns is obtained best by the comprehensive Grob-test (Grob, K., Grob, G. and Grob, K. jr., 1981). Since the capillary columns described in this study are preferentially used for high temperature applications, their properties, particularly in the catalytic sense, should be tested at elevated temperatures.

The only suitable test procedure published so far is that introduced by Donike (Donike, M., 1973) for packed columns. The Donike test can be easily adapted to capillary columns and extended to higher temperatures (Blum, W., 1985).

The extended "apolar" Donike test utilises the TMS-esters of the following fatty acids: capric acid (C_{10}, myristic acid (C_{14}), stearic acid (C_{18}), behenic acid (C_{22}), hexacosanoic acid (C_{26}), melissic acid (C_{30}), and the n-alkanes: hexadecane (C_{16}), eicosane (C_{20}), tetracosane (C_{24}), octacosane (C_{28}), dotriacontane (C_{32}) and hexatriacontane (C_{36}).

Since the test components of the "apolar" Donike test mixture are not fully separated on the OV-225-OH coated column the "polar" Donike test mixture was used, again extended to higher temperatures (Blum, W., 1986b).

The test utilises the TMS esters of the following fatty acids: myristic acid (C_{14}), stearic acid (C_{18}), behenic acid (C_{22}), hexacosanoic acid (C_{26}), melissic acid (C_{30}), and the n-alkanes: tetradecane (C_{14}), octadecane (C_{18}), docosane (C_{22}), hexacosane (C_{26}), triacontane (C_{30}), and tetratriacontane (C_{34}).

All test components are available from Fluka AG, CH-9470 Buchs, Switzerland.

100mg of each test component is diluted in 80ml of a mixture of pyridine/hexane = 1:1 and made up to a volume of 100ml with 20ml of N-methyl-N-trimethylsilyltrifluoroacetamide (MSTFA, Pierce, Box 117, Rockford Illinois 81105, USA). An aliquot of this stock solution containing 1mg/ml of each component is diluted with a mixture of hexane/MSTFA 50 x for an on-column injection.

VII.2.8.1. Test Results

Whereas the Grob test serves for general evaluation of the column and determination of the basic parameters, such as separation efficiency, polarity of the stationary phase, adsorptive activity, etc., the main information which is forthcoming from the Donike test is the so-called (temperature dependent) catalytic activity factor for the temperature range above 150°. All phases used in this study were tested with the Grob- and the Donike test prior to porphyrin analysis.

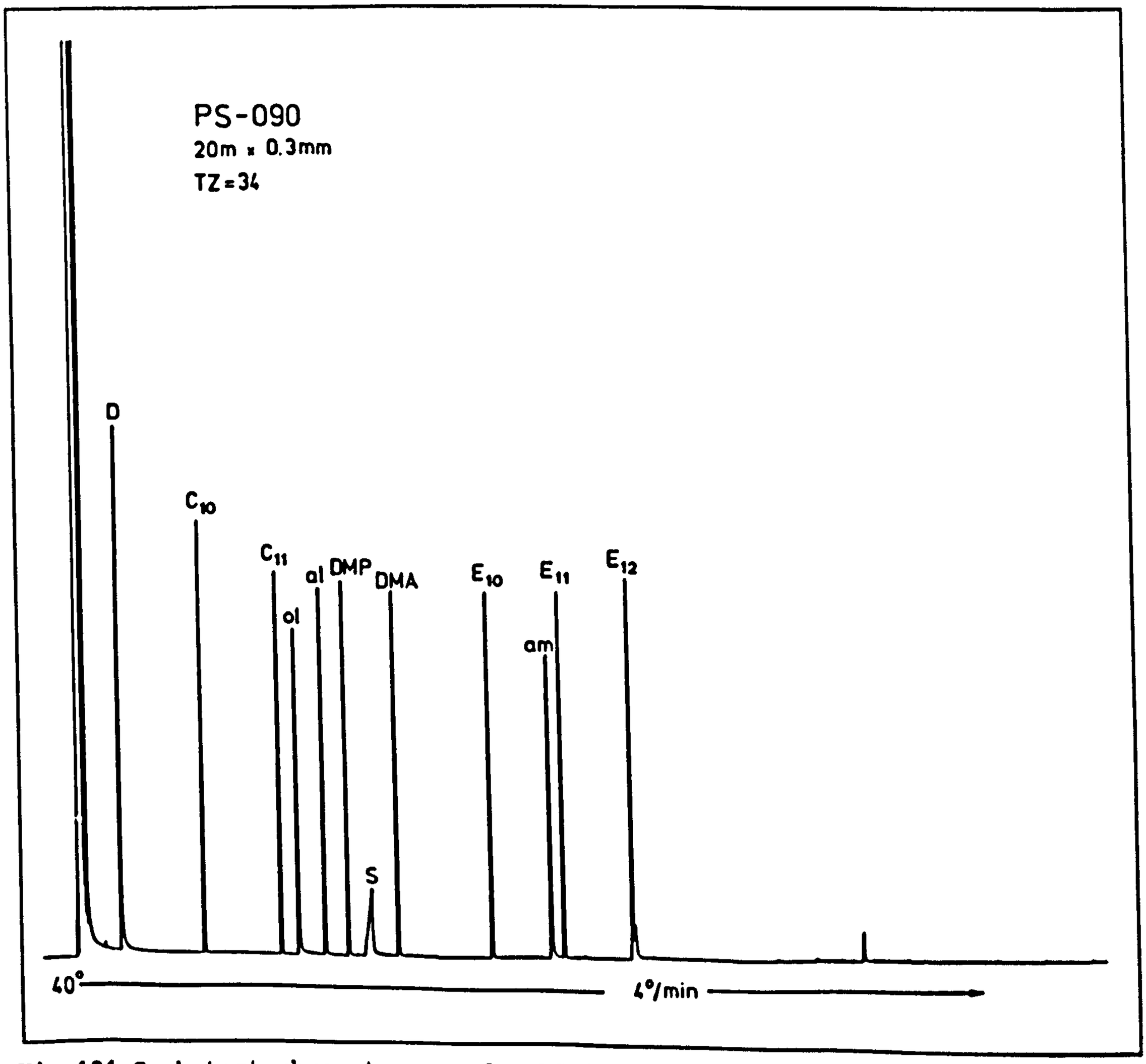

Fig.101 Grob test chromatogram of a PS-090 coated column. D = 2,3-butanediol, C_{10} = n-decane, C_{11} = n-undecane, ol = 1-octanol, al = nonanal, DMP = 2,6-dimethylphenol, s = 2-ethylhexaconoic acid, DMA = 2,6-dimethylaniline, E_{10} = methyl decanoate, E_{11} = methyl undecanoate, am = dicyclohexylamine; E_{12} = methyl decanoate.From Blum and Eglinton, 1989

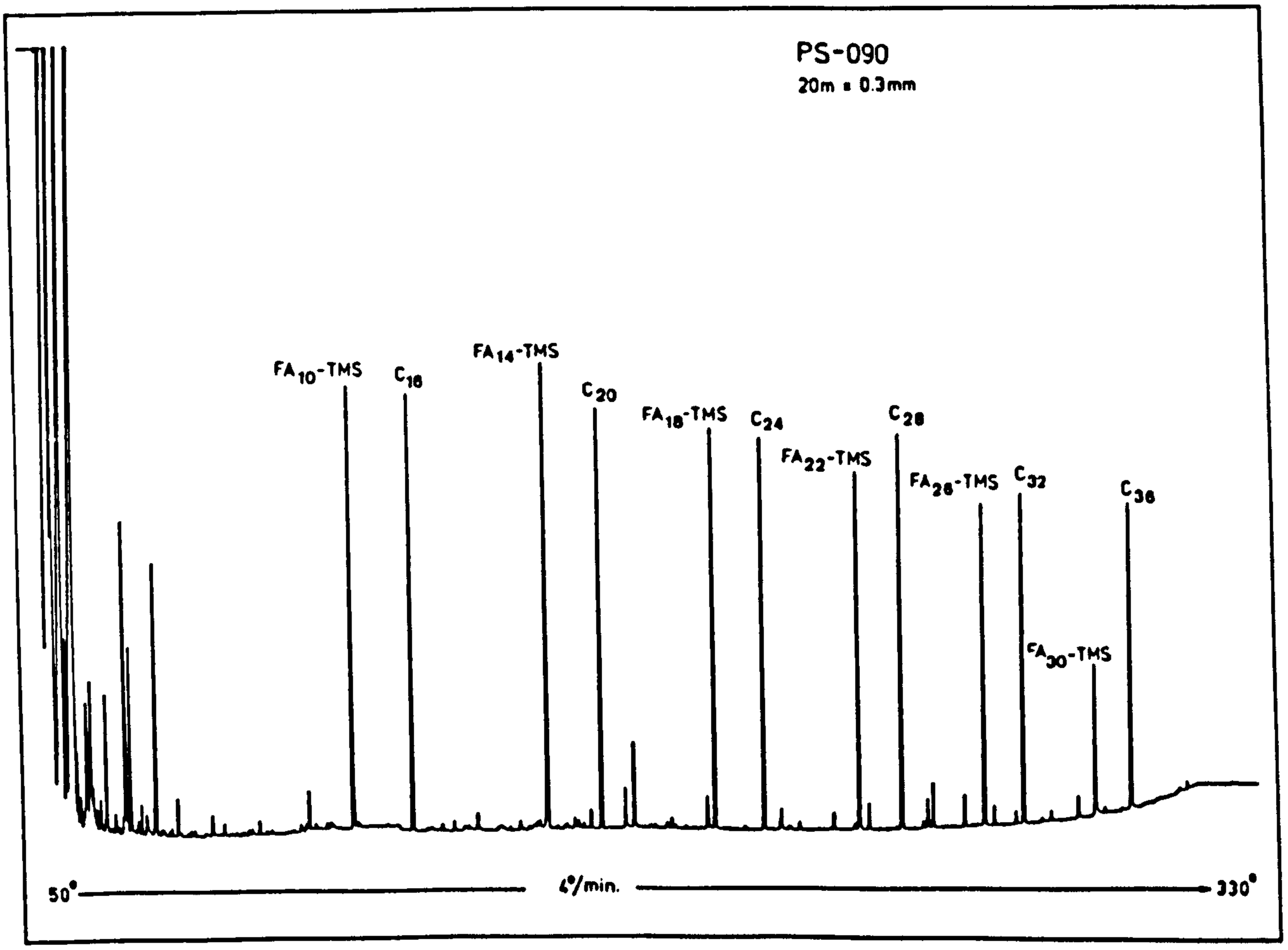

Fig.102 Donike test chromatogram of a PS-090 coated column. FA_{10}-TMS, FA_{14}-TMS, FA_{18}-TMS, FA_{22}-TMS, FA_{26}-TMS and FA_{30}-TMS = TMS-esters of capric acid (C_{10}), myristic acid (C_{14}), stearic acid (C_{18}), behenic acid (C_{22}), hexacosanoic acid (C_{26}) and melissic acid (C_{30}). C_{16} = hexadecane, C_{20} = eicosane, C_{24} = tetracosane, C_{28}= octacosane, C_{32} = dotriacontane, C_{36} = hexatriacontane. From Blum and Eglinton, 1989.

In contrast to the Grob test, where activity is deduced from the tailing behaviour of some polar test components, the Donike test provides a quantitative description by the quotient:

$$Q = \frac{A_{fa} - TMS}{A_{alk}}$$

where Q is the activity factor, A_{fa}-TMS the peak area of the fatty acid-TMS ester, and A_{alk} the peak area of the corresponding n-alkane. All Q-values <1 imply activity.

As shown in the Grob test chromatograms, the polarity of the coated CH_3O-terminated phase after condensation is the same as that of the corresponding conventional pendant. This leads to the conclusion that no excessive reactive functions are left in the stationary phase.

Although some phenyl containing stationary phases with relatively high surface tension like OV-61-OH, which need a highly active surface for a sufficient distribution, show some tailing of OH-containing test-components, particularly of 2,3-butanediol (Blum, W.,1985), this behaviour is not reflected in the Donike test. On the other hand , the cyanoalkyl-containing polymers such as OV-225-OH from which the components of the Grob test elute nearly perfectly show some activity from the beginning of the test and increased activity above a column temperature of 250°C (Blum, W., 1986).

While we have no explanation for the behaviour of the pheny-containing phases (eventually an impurity in the polymers), the behaviour of the cyanoalkyl substituted phases can be interpreted in the sense that some catalytic activity could be induced by the cyanoalkyl function. The basic activity of cyanoalkyl-containing stationary phases was explained by the presence of trace amounts of amides as the first saponification product of cyano groups and of carboxy groups resulting from further hydrolysis (Blum, W., 1986). The greater the amount of cyano functions in the polymer, the higher the catalytic activity. This behaviour was quantitatively reflected by the Donike test.

VII.2.9. Repair of Contaminated Column Entrances by High Temperature Stable Fused-Silica Glass Press-Fit Connections

In order to prolong the life time of capillary columns contaminated at the entrance region, in some cases the first coils of a column were replaced by fused-silica pieces. For this purpose the press-fit technique described by Rohwer (Rohwer,E.R. et al.,1986) was modified for high temperature work.

Press-fit connections in the butt of glass capillary columns re-

quire the formation of a conical seat of the dimensions suiting the outer diameter of the fused-silica capillary to be attached. The conical seat is formed by using a tungsten point as reported by Grob et al. (Grob, K. jr., et al., 1988). Tungsten points of 0.5 and 0.7mm diameter are available from Brechbühler AG, CH 8952 Schlieren, Switzerland.

The tungsten point must be turned during preparation of the tapered seal, on one hand to avoid adhesion of the glass and on the other hand to ensure the formation of a concentric seat. For this purpose the glass is heated indirectly, by heating the tungsten point with a small flame slightly behind its tip to render the point red hot.

During the widening process, the sharp edges of the freshly cutted glass capillary butts are flame polished. No further precautions are necessary to avoid the damage of the polyimide coating of the fused-silica capillary.

Preparation of the conical seat is facilitated by a "column end widener" GWM-87 also available from Brechbühler AG. The apparatus includes a boring jig turning the tungsten point at 52rpm around a stable axis and moreover keeping the flame in a stable position. High temperature stable butt-connections are produced by choosing high temperature stable fused-silica capillaries with an outer diameter of 0.3mm, which can be used at 450°C for 50h (Siemens) and secondly by heating the press-fit with a hot air fan to about 550°C. During this treatment, combined with the strong lateral forces exerted by pushing the fused-silica capillary butt into the conical glass seat, the thin layer of polyimide is amalgamated with the softened glass surface to a ferrule providing both mechanical stability and tightness of fit, even at temperatures above 400°C.

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